MINERAL ELEMENTS AND EMBRYO DEVELOPMENT IN BARLEY

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The work contained in this thesis is my own original work and has not been submitted for a degree at any other University.

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CONTENTS

Abstract

This work describes the accumulation of a number of essential mineral elements during embryo development in barley together with the effects of variation in their supply on embryogenesis.

The mineral elements investigated were potassium, magnesium, calcium, iron, copper and manganese, and a rapid wet ashing procedure, coupled with flame and flameless atomic absorption spectroscopy was devised for their determination in barley embryos and endosperms. Recoveries of all the elements studied from embryo and endosperm samples were good.

Although the accumulation of all these mineral elements was found to be related to embryo growth, the concentrations (w/w) were much higher at earlier, rather than later, stages of development. For example almost half the calcium content of the mature embryo was assimilated by 21 days after anthesis.

The patterns of mineral ion accumulation in four barley cultivars were similar, although the concentrations (w/w) of calcium were rather higher in the cultivar Julia.

Variation in the magnesium supply led to higher manganese and calcium, and lower magnesium concentrations (w/w), in the developing embryos. Manganese did not totally compensate for lack of magnesium and chlorosis was observed in seedlings from magnesium 'deficient' seeds. Only at later stages of development did the endosperm become deficient in magnesium.

The withdrawal of both magnesium and manganese from the rooting medium resulted in low magnesium levels in both the embryos and endosperms. As before, the embryo manganese levels increased, but in this case the endosperm manganese levels fell. Potassium levels in both cases were unaffected.

In vitro embryo culture was also used to investigate the effects of variation in the mineral supply on embryo development. Norstog's Medium II (0.2 M sucrose) satisfactorily supported the growth of barley embryos in culture, as assessed by a number of criteria including embryo ultrastructure and external morphology. Magnesium withdrawal resulted in a significant increase in embryo size. Whether this was related to cell number or cell size was not determined.

The results are discussed both in relation to the biochemistry and physiology of embryogenesis and to the possible role of the endosperm in the nutrition of the growing embryo.

ABBREVIATIONS

AAS	=	Atomic absorption spectroscopy and Atomic absorption spectrophotometer
ATP	=	Adenosine triphosphate
CRA	=	Carbon rod atomiser
DNA	=	Deoxyribonucleic acid
d. weight	=	dry weight
f. weight	=	fresh weight
lxw	=	length x width
NAD	=	Nicotinamide adenine dinucleotide
RNA	=	Ribonucleic acid
v/v	=	unit volume per unit volume
w/v	=	unit weight per unit volume
w/w	=	unit weight per unit weight

1.0 General Introduction

1.1 Growth of the barley plant

Following germination, the first leaf emerges from the coleoptile tip. The upper part of the stem gradually gives rise to the crown. Adventitious root initials then form which subsequently give rise to the tillers. The ear primordia are produced when the apical primordia cease to produce leaf initials. The last leaf or 'flag' leaf contains the ear within its sheath (boot). Anthesis generally takes place soon after ear emergence but under some circumstances can take place within the sheath. Senescence occurs with mature leaves and gradually the whole plant dries out when the grain is ripe.

The duration of the different developmental stages varies widely, depending on the geographical area of cultivation, the time of sowing and the variety being grown. In the U.K. spring sown barley requires 120 - 140 days from sowing to maturity, while in western continental Europe it requires about 120 days and 100 days in North Europe (Briggs 1978). In contrast autumn grown barley in the U.K. may take up to 270 days to reach maturity (Bergal and Clemencet 1962).

1.2 Tillering

During initial growth the tillers are entirely dependent on the shoot for the supply of carbohydrates and other nutrients, but become independent after the formation of adventitious roots. The mineral nutrient supply to the barley plant is one of the factors that influences the peak number and chances of survival of tillers (Aspinall 1961). In the field the crown typically carries several culms, a main stem and two or three tillers (Briggs 1978), but barley plants grown under controlled environment conditions with a continuous supply of nutrients may bear up to 120 tillers per plant (Aspinall 1961).

1.3 The mature barley grain

The barley ear consists of spikelets on each side of the axis, each spikelet containing only one flower. The palea (palea superior) and lemma (palea inferior) act as a protector to the flower and the 'glumes' protect the spikelet. Fig. 1.1 shows the different tissues and their position in a mature barley grain. In most varieties, the glumes, lemma and palea adhere to the grain and the caryopsis is bound within. The remains of the ovary wall, the pericarp, is fused to the testa and the starchy endosperm, bounded at the periphery by the aleurone layer, lies within. At the basal end of the grain lies the embryo.

1.3.1 The embryo

The cereal embryo is a complex structure and interpretation of the nature of different regions of the embryo is subject to controversy (Brown 1965), and there is not always agreement among botanists as to the nature of the different regions. Fig. 1.2 shows the different structural regions of an embryo from a mature cereal grain. The four 'distinct' regions are (1) the root system, with a primary root covered by the coleorhiza; (2) the acrospire, which includes the coleoptile stem apex and leaf primordia; (3) the nodal region between root and shoot; and (4) the scutellum.

The morphology of the scutellum has been the subject of much debate. Various views have been presented, (Merry 1941, Jacques-Félix 1957, Negbi & Koller 1962). For example, the scutellum maybe the first 'embryonic' leaf, or possibly the root and scutellum may together constitute the true embryonic axis where the apical shoot meristem is a lateral bud. Brown (1965) concluded on the contrary, that the scutellum was not homologous with any structure of mature plants, and together with the coleoptile and mesocotyl had evolved as useful and peculiar to the embryo alone.

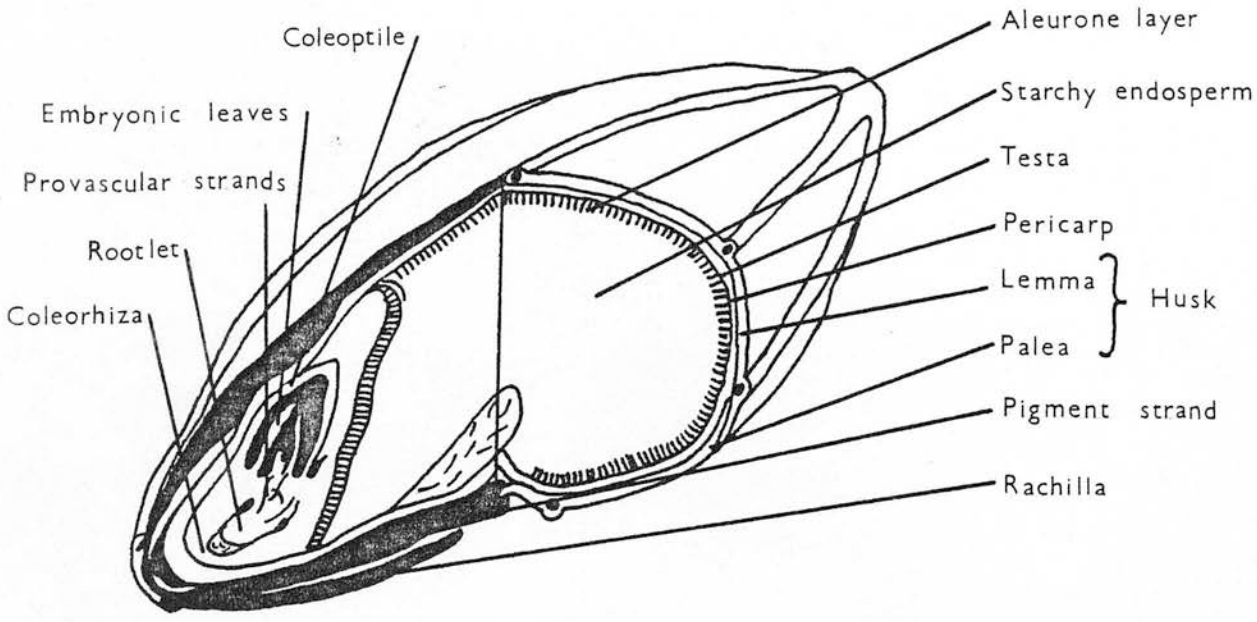


Fig.1.1. IDEALIZED DIAGRAM OF A BARLEY GRAIN (Briggs 1978)

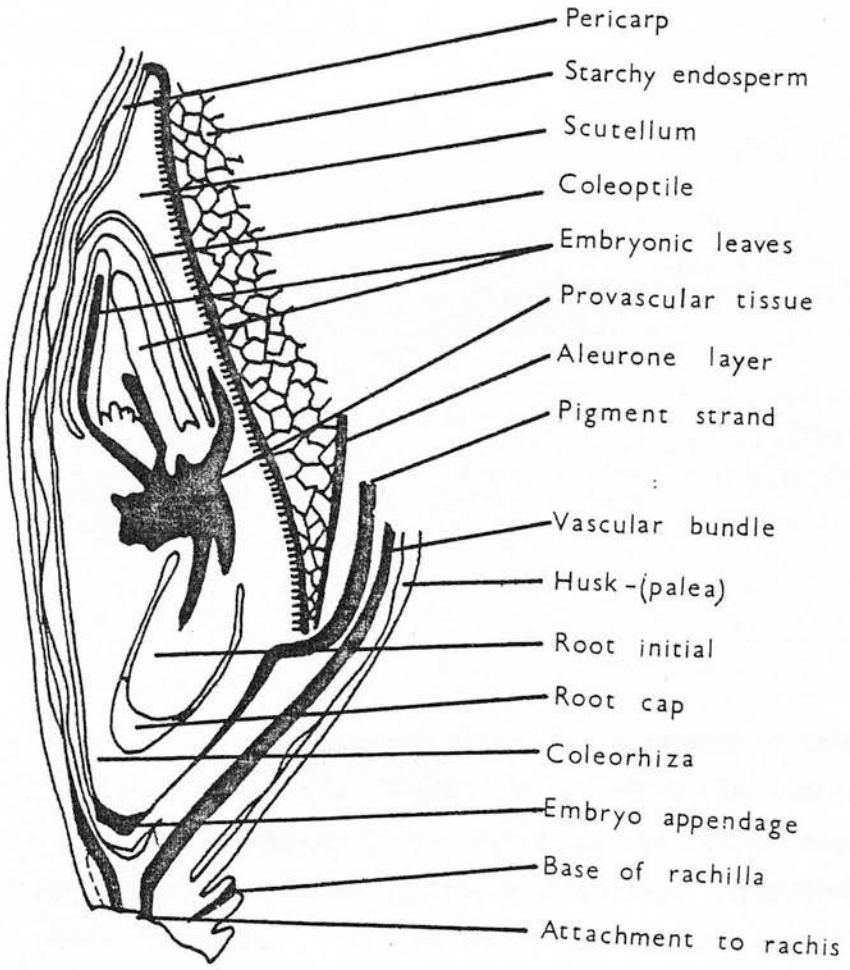


Fig.1.2. THE EMBRYO IN LONGITUDINAL SECTION (Briggs 1978)

However, since it is difficult to separate the scutellum physically from the organogenetic parts, the embryo in most studies with monocotyledonous seeds, is considered to contain the organogenetic parts as well as the scutellum.

1.4 Grain development

The initial events following fertilization have been well described (Brink and Cooper 1947, Loxová 1967). The two male gametes are carried to the ovary i.e. the tip of the pollen tube. The nucleus of one fuses with the nucleus in the egg cell forming a zygote, whilst the other enters the central cell and unites with two polar nuclei to form the triploid primary endosperm nucleus. Rapid division of the triploid endosperm nucleus follows, the rate of cell division exceeding that of the zygote which later gives rise to the embryo. By about ten days after anthesis the barley endosperm represents 3 per cent of total grain fresh weight.

1.4.1 Morphological changes

Changes in the ultrastructure of endosperm during grain development have been desribed in some detail for barley and wheat (Buttrose 1960, 1963). Mitochondria, proplastids, endoplasmic reticulum and Golgi bodies were observed as early as five days after anthesis. Amyloplasts and protein bodies were distinguishable in barley endosperm 18 days after anthesis (Duffus 1979).

The aleurone cells which surround the endosperm, apart from the area adjacent to the embryo, could be distinguished in wheat about ten days after anthesis (Morrison *et al.* 1975). The aleurone layer was one cell thick in wheat and three cells thick in barley (Jacobsen *et al.* 1971). At maturity the cytoplasm of these cells contained numerous aleurone grains (protein granules) completely surrounded by lipid droplets. Ultrastructural changes in the embryo during development have also been studied (see below) although less thoroughly probably due to difficulties arising from small embryo size.

The maternal tissues that surround the embryo and endosperm, the testa pericarp, glumes and palea also undergo changes during grain maturation. In cereals, apart from the testa, these tissues contain chlorophyll initially, which is then lost during later stages of development. As a result of the role of the pericarp in carrying out photosynthesis (Duffus and Rosie 1973, Radley 1976), some attention has been paid to describing its structure (Morrison 1976, Cochrane and Duffus 1979). The surface of the wheat and barley pericarp, 15 - 30 days after anthesis when examined by scanning electron microscopy showed the presence of stomata on the ventral side at the apical end. The outer epidermis contained elongated cells which were three times as long as broad. The inner layers of the pericarp comprised of cross-cells, containing chloroplasts and tube cells. Starch was present in the chloroplasts ten days after anthesis to the point where the grain turned from bright green to yellow.

1.4.2 Biochemical changes

The most important change that occurs during grain development is the deposition of starch in the endosperm. The overall changes in accumulation of storage material such as starch, nucleic acids, lipid and protein have been described by Jennings and Morton (1963 a, b). For example, the starch content of cereal grains increased steadily throughout development and represented around 65-75 per cent of grain dry weight at maturity. The carbohydrate metabolism of cereal grain during development has been reviewed (Duffus 1979). The conversion of sucrose to starch in sweet corn (de Fekete and Cardini 1964), rice (Perez *et al.* 1975) and barley (Baxter and Duffus 1973) was attributed to UDP - dependent sucrose synthase which has relatively high activity. In this context the mechanism of sucrose utilization in the developing embryo appears to be slightly different (Duffus and Rosie 1975). Invertase activity was present in significant amounts very early on in development (18 days after anthesis) and the ADP - and UDP - dependent sucrose synthase could only be detected 21-25 days after anthesis. Thus it is possible that in the very early stages some sucrose is cleaved by invertase during entry and diffuses as monosaccharides to the embryo cells. Since cereal grains may account for a substantial amount of gross ear photosynthesis (Evans & Rawson, 1970), the

photosynthetic properties of the pericarp during grain development have been investigated (Duffus and Rosie 1973, Nutbeam and Duffus 1976). The first formed product of photosynthesis in the pericarp was shown to be the C_4 acid malate which was subsequently converted to sucrose. Interestingly this tissue showed some of the characteristics of C_4 plants (Nutbeam and Duffus 1976).

The respiratory metabolism in the developing barley endosperm was indicated by the presence of a number of enzymes of the Embden-Meyerhof-Parnas and pentose phosphate pathways (Duffus & Rosie 1977). The activities of these enzymes were initially low but rose with time reaching a maximum at approximately half way through the development of grain. The enzymes of the pentose phosphate pathway maintained quite high activity in the later stages of development, whilst those of Embden-Meyerhof-Parnas pathway fell to low levels at maturity.

1.5 Embryo development

During development of the embryo, cell division, cell expansion, cell maturation, cell differentiation and formation of meristem take place. The plane of cell division and the relationship between the frequency of cell division and the rate and direction of cell elongation may determine the changes leading to transformation in size and shape of the embryo. Hence embryogenesis can be regarded as the continuum of processes involved in the origin, growth and orderly transformation of the zygote into a fully fledged embryo (Wardlaw 1955).

1.5.1 Morphological changes

Wardlaw (1955) has described the gross morphological changes during the early stages of embryo development in higher plants. Detailed morphological development of the barley embryo, Hordeum sativum, from the time of fertilization to the maturation of the ovule into a seed have been recorded using light microscopy (Merry 1941). The embryo was observed to grow from the apex to the base. The important points noted were: (1) that the pro-embryo developed with no apparent pattern of cell division, (2) the scutellum and stem meristem began to differentiate eight days after fertilization, (3)

the coleoptile was formed from the region which later becomes the scutellum and (4) the time of differentiation of the seminal-root primordia was given. It was suggested that due to apparent irregular cell divisions, the factors controlling the growth of the embryo probably affect the embryo as a whole rather than at the level of individual cells.

The ultrastructural changes during the very early stages in embryogenesis (up to 20 celled stage) in barley have been described (Norstog 1972). Although the planes of cell division tended to be irregular, a pattern of cell disposition was discerned. A basal suspenser cell was seen to 'anchor' the embryo during early embryogenesis, but by about five division cycles the embryo lost its attachment to the nucellus and was completely surrounded by the endosperm.

This is one of the few reports on ultrastructural changes occuring during barley embryogenesis, more attention being paid to the fine structure of the mature embryo (Setterfield *et al.* 1959, Swift & O'Brien 1971). However, some complete descriptions of the fine structure of developing plant embryos are available, particularly those of Capsella (Schulz & Jensen 1968 & 1969, Sec.11.0)

1.5.2 Biochemical changes

The hypothesis that metabolic changes and regional differences in biochemical properties are linked to morphogenesis, although quite well established in animal embryology, is not well understood in plant embryos particularly those from monocotyledonous plants.

The pattern of nucleic acid changes during embryogenesis has been investigated to some extent. In cotton embryos, the amount of DNA per cell remained fairly constant during embryogenesis (Fisher and Jensen 1972). Very little extranuclear DNA was present. However, an earlier study implied the presence of cytoplasmic DNA in the young embryo, and a dramatic decrease in DNA content per cell as the embryo matured (Yoo and Jensen 1966). The reasons for these different observations were not clear. The DNA content per embryo was found to be constant in barley embryos during the later developmental stages (Duffus and Rosie 1975). The significance of this observation would be greater had the data been expressed on a per cell basis.

Changes in the RNA content of embryo cells may provide some explanation as to the origin of functional differentiation in developing embryos (Raghavan 1976). However, there is no definite evidence for this as yet. The RNA content of cotton embryos increased with increase in embryo size, but RNA content per cell decreased with progressive embryogenesis (Yoo and Jensen 1966 Fisher and Jensen 1972). Similar results using ^{32}P incorporation into nucleic acids in barley embryo showed an increase in the absolute amount of incorporation of ^{32}P into RNA, but incorporation into RNA per cell per unit time decreased (Chang 1963a). These results may indicate that the capacity of the nucleus to synthesize new RNA does not keep pace with the rapid cell multiplication that occurs during embryogenesis. The RNA content per embryo was shown to increase rapidly at early stages of embryogenesis reaching a maximum at maturity (Duffus and Rosie 1975).

The changes in total protein content of wheat and barley embryos during development have been examined (Jennings & Morton 1963 a, Duffus and Rosie 1975). Protein content increased steadily during wheat embryo development; barley embryos showed a rapid accumulation in the initial developmental period, reaching a maximum at about fifty days after anthesis. Similar rapid increases were observed in developing maize embryos (Ingle 1965). Cereal embryos contain protein as the main storage material in contrast to carbohydrate in the endosperms. The storage protein was present in protein bodies which showed considerable structural diversity (Lott & Buttrose 1978, Lott & Spitzer 1980).

Few reports are available on the changes in carbohydrates during development of cereal embryo. Jennings and Morton (1963 a) have described the changes in carbohydrate composition of developing endosperm and testa pericarp but the embryo was not investigated.

In developing barley embryos the level of total carbohydrate was initially low, but appreciably higher than reducing sugars. Rapid accumulation occurred between 18 and 45 days when a maximum was attained. It was suggested that some of this carbohydrate was likely to be starch (Duffus and Rosie 1975). Both wheat (Swift & O'Brien 1972) and barley (Nieuwdorp 1963, Palmer 1967) scutella apparently contain no amyloplasts and presumably therefore, no starch. Levels of reducing sugars were found to remain low throughout barley embryo development (Duffus & Rosie 1975).

The synthesis of protein and carbohydrate requires metabolic energy which may be provided by oxidative pathways involved in respiration. Once again not much is known about the process of respiration associated with embryogenesis. Forman & Jensen (1965) reported the first quantitative information for the early developmental stages. Respiration rates as measured by oxygen uptake showed that uptake increased as cotton embryos increased in size. However, when oxygen uptake was averaged on a per cell basis, the amounts decreased with development. Using inhibitors, it was found that the tricarboxylic acid cycle pathway appeared to be the only pathway involved in the oxidation of substrates to carbon dioxide.

1.6 Mineral nutrition

1.6.1 Definition of essential element

For a mineral element to be classified as essential it must fulfil three main criteria (Arnon & Stout 1939, Arnon 1950):

1. If the element is removed from the growth medium of a plant, the plant must be unable to complete its vegatative or life cycle.

2. If its function is specific and cannot be replaced by other elements.

3. The essential element must have a direct effect on some aspect of growth or metabolism.

However, there are other points to be considered - is an element essential if it removes the detrimental effects of another element present in excessive amounts? If a so-called essential element is present in insufficient amounts, its function may be partially replaced by another element. It is also important to note that certain elements may not be essential for any species, that some may be required quite specifically by some species and not by others, and that some may be required only under certain conditions imposed by the nutrient environment (Hewitt & Smith 1975).

1.6.2 Classification of essential elements

Table 1.1 - lists all of the elements considered to be essential for higher forms of plant life and the levels known to be critical for growth.

Table 1.1 Levels of essential elements known to be critical for growth of multicellular plants.
(After Stout (1961) as presented in part by Price (1970)

Element	Concentration in dry matter $\mu g\ g^{-1}$
Major elements	
Carbon	450,000
Oxygen	450,000
Hydrogen	60,000
Nitrogen	15,000
Potassium	10,000
Calcium	5,000
Magnesium	2,000
Phosphorous	2,000
Sulphur	1,000

Table 1.1 continued

Trace elements	$\mu g\ g^{-1}$
Chlorine	100
Iron	100
Manganese	50
Boron	20
Zinc	20
Copper	6
Molybdenum	0.1

All the elements present at concentrations of 1,000 $\mu g\ g^{-1}$ or higher are defined as major elements or macronutrients. The others in the table are termed trace elements or micronutrients. Despite the small amounts required they are no less essential than the major elements. The identification of major elements has been easier since they are required at much higher concentrations as compared to the trace elements.

1.6.3 Beneficial elements

Addition of a particular element to the soil or culture solution may lead to improved growth without that element being necessarily essential. Rubidium (Rb), Strontium (Sr), Cobalt (Co), Selenium (Se), Aluminium (Al) and Silicon (Si), are among the elements which have been shown to have such beneficial effects. In some cases e.g. Rb and Sr, the element may substitute in part for an essential element, in this case K or Ca, and thus produce beneficial effects when this element is deficient. Alternatively, the beneficial element may stimulate absorption or transport of an essential element which is in limited supply, or conversely inhibit uptake and distribution of one that is in excess.

1.6.4 Functions of essential elements

It is possible to generalise as to the function of essential major elements. By making K the obvious exception, one can assign a role in either the structure of plants or as components of plant metabolites (Epstein 1965, Evans & Sorger 1966, Hewitt 1963). This probably accounts for the relatively high requirements for these elements in plant metabolic processes.

There is a vast amount of literature on the specific functions of essential nutrients. Only a brief summary is presented here, based on information from the following sources: Hewitt (1963), Hewitt & Smith (1975), Nason & McElroy (1963), Epstein (1965, 1972), Evans & Sorger (1966), Rains (1976), Clarkson & Hanson (1980). The table below only includes those elements studied in the present investigation.

Potassium	Magnesium
Osmotic regulation, Translocation of sugars, Stomatal opening. Some enzyme systems requiring K:- Acetic thiokinase, Pyruvate kinase, Phosphofructo-kinase, Succinyl CoA synthetase, Nitrate reductase, granule bound Starch synthetase. Activation of ATPase systems.	Requirement in chlorophyll structure. Ribosome integrity. Binding transfer RNA to ribosomes. Some enzyme systems requiring Mg:- Involved in transfer reactions with phosphate-reactive groups. Activating almost every phosphorylating enzyme in carbohydrate metabolism. Ion transport mediated by ATPase:- activation of K-Na-stimulated ATPase; active uptake of Ca requires Mg-ATPase.

Calcium

Cell wall structure - calcium pectate. Influences non-specific growth responses. Membrane structure and ion fluxes. Protective role in ion transport and other physiological processes. Nodulation and nitrogen fixation.

Some Enzyme systems requiring Ca:- α-Amylase, ATPase, Phospholipase D, influences Nitrate reductase.

Manganese

Important role in photosynthesis. Electron transport in photosystem II. Maintenance of choloroplast membrane structure.

Some enzyme systems requiring Mn:- can replace Mg in many of the phosphorylating and group transfer reactions. Many of the enzyme reactions in citric acid cycle require Mn for maximal activity. Chromatin-bound RNA polymerase. NAD malic enzyme of aspartate-type C_4 plants has an absolute specific requirement for Mn.

Iron

Structural component of porphyrin molecules, cytochromes, hemes, hematin, ferrichrome. Involved in oxidation-reduction reactions in respiration and photosynthesis. Structural component of non-heme molecule ferredoxin. Role in cell division and growth.

Some enzyme systems requiring Fe:- Cytochrome oxidase, Catalase, Peroxidase, Aconitase, Nitrogenase.

Chlorophyll synthesis; γ-aminolevulinate dehydratase and γ-aminolevulinate synthetase, ferrochelatase.

Copper

Oxidase enzyme; tyrosinase. Terminal oxidation by cytochrome oxidase. Photosynthetic electron transport mediated by plastocyanin. Indirect effect on nodule formation.

1.6.5 Mineral ions in developing grain

The failure of plants to grow normally and the deficiency symptoms that arise from metabolic disorders in the vegetative growth phase have received much attention. The effects of soil mineral deficiency on grain growth and yield has not been widely investigated and little is known of the role of essential mineral elements during seed development. Additionally, the role of mineral ions in seeds is extended to include storage for use in subsequent germination and growth.

Changes in nitrogen, ash and phosphoric acid were followed during maturation of barley grain from plants grown in three plots under deficient nitrogen, phosphorus and plentiful nutrient condition (Brenchley 1912). The actual amounts in the whole grain increased as the grain matured. The ratios between nitrogen and phosphorus in the grains showed that lack of phosphorus resulted in a larger proportion of nitrogen in the grains. It was postulated that phosphorus might affect the transport of nitrogen from the soil. Although these experiments formed the basis of results obtained later, where macronutrient deficiencies affect the nitrogen status of the plant (Hewitt 1963), the interpretation of results is questionable due to the undefined nature of the soil used.

Non-protein nitrogen was found to decrease in concentration during development of wheat grain from a few days after flowering to maturity. (Jennings & Morton 1963 a). Changes in total nitrogen content of whole wheat grain as well as the separated component parts were investigated. Total endosperm and embryo nitrogen as a per cent of total grain nitrogen, reached a peak value at maturity. Changes in phosphorus content in endosperm and testa pericarp

increased fairly rapidly from five days after flowering to maturity and in the embryo from fifteen days up to maturity (Jennings & Morton 1963 b). In the endosperm and testa-pericarp there was a relationship between the content of inorganic phosphorus, acid labile phosphorus and acid soluble organic phosphorus (phytic acid), and the water content of the grain. As the phytic acid content increased there was a decline in the content of inorganic phosphorus.

Little information has been available on the other essential major elements K, Na, Ca, Mg and micronutrients in the developing cereal grains. The very small concentrations in the seed and seed parts posed difficulties in detection. With the improvement in analytical sensitivity, the levels of these essential elements have been determined. The deposition of K, Ca, Mn, Fe, Cu, Zn, and Br in wheat, rye and triticale grains was investigated from early grain development to full maturity using energy dispersive x-ray fluorescence spectrometry (Lorenz & Reuter 1976). Results indicated that deposition commenced at the initial stages of kernel development. Changes in the amount of N, P, Mg, Ca, Na, Fe, Zn and Cu in various grain tissues of developing barley grains have been reported (Duffus & Rosie 1976 a, b). An overall pattern of accumulation was observed in all the tissues.

Although the distribution of elements in different grain tissues can be determined with some accuracy, the distribution within the tissues has been difficult to determine. The studies of possible tissue-to-tissue and cell-to-cell variation in mineral contents may be important for an understanding of how cereal grains develop and subsequently germinate.

X-ray analysis (electron microprobe x-ray analysis (EMX) and energy dispersive x-ray analysis (EDX) in conjunction with scanning and transmission electron microscopy have been used in several investigations on mineral studies in seeds. This system has been chosen for several reasons and some of the advantages include : the capacity to spot analyse chosen cell regions; a high detection sensitivity of 10^{-17} to 10^{-18}g (Russ 1972); the ability to analyse simultaneously all elements of interest including P, K, Mg, Ca, Fe and Mn, (Lott & Spitzer 1980)and the capacity to analyse very thin sections of tissue in the electron microscope.

Almost all the work has been carried out on mature seeds and emphasis placed on storage in seed tissues. Mineral reserves have been reported to occur mainly within the elctron-dense globoid crystal portion of protein bodies from a number of plant seeds including those of cucumber, cotton, rice, castor, wheat, barley, rye and oats (Lott 1975, Lui & Altschul 1967, Ogawa *et al.* 1975, Surorov *et al.* 1971, Lott & Spitzer 1980,Liu & Pomeranz 1975).These mineral reserves seem to be mainly in the form of phytin, a cation salt of inositol hexaphosphoric acid (Ashton & Williams 1958, Ergle & Guinn 1959, Tanaka *et al.* 1973). While Mg and K are the most commonly occurring cations found in globoid crystals, a range of other ions including Ba, Ca, Fe, Mn and Na can also be present (. Lott & Spitzer 1980).

The mineral reserves of cereal grains have received some attention. For example, selected tissues of rice grain have been studied with EMX or EDX. P, K, Mg, Fe and Mn were found to be concentrated in the aleurone layer with P, Mg, and K predominating. By contrast, Ca was abundant in the pericarp (Tanaka *et al.* 1974 a). The rice scutellum cells were also found to contain high contents of Mg and K salts of phytic acid (Tanaka *et al.*1976, 1977).

Similarly, x-ray analysis of mature wheat grains shows that P, K, and Mg were concentrated in aleurone bodies (Tanaka *et al.* 1974 a). Lott & Spitzer (1980) have also described the distribution of elemental storage in wheat protein bodies. K and Mg were concentrated in the aleurone layer but unlike Tanaka *et al.* (1974 b) only traces of Fe and no Mn was detected. Ca was occasionally found in globoid crystals in the aleurone layer. No protein bodies with electron dense globoid crystals were found in the starchy endosperm. Additionally in barley, Ca and P levels were found to be higher in the inner, than in the outer layer of the lemma, and the two main components in the pericarp as with rice, were K and Ca (Liu & Pomeranz 1975). The barley aleurone layer had a mineral composition similar to that of wheat and rice. However unlike the latter two, Ca could be detected in the aleurone grains of barley seeds.

Although x-ray analysis coupled with electron microscopy appears to have considerable advantages, the technique has limitations, particularly that of migration of elements during the preparation of tissue for electron microscopy. Thus the technique in its present stage is only qualitative and to date has only been suitable for mature seeds. In addition to migration of elements during preparation of samples, highly localised concentration of elements is required for detection.

1.6.6 Mineral elements in the embryo

The distribution and role of mineral ions in plant embryos during development has received even less attention. One of the few reports on mineral nutrition during cereal embryogenesis is that of Jennings & Morton (1963 a, b). Both N and P accumulation in developing wheat embryos increased with increase in dry weight of embryos. The embryos had a relatively high P content and embryo P

contributed substantially to the total P content of the whole grain. Studies on major and trace elements in developing barley grains also involved the analysis of N, P, Mg, Ca, Na, Fe, Zn, Mn and Cu during embryo development (Duffus & Rosie 1976 a, b). There was a steady accumulation of the mineral elements as the embryo developed. The accumulation of Mn was unusual in that it was undetectable in the embryo until 45 days after anthesis, when levels rose dramatically to reach a steady maximum. The results were discussed in relation to known biochemical events accompanying embryo maturation.

Within the dry embryos, mineral elements are also located in the globoid crystals which normally occur within storage protein bodies (Lott 1975, Lott & Vollmer 1979). Once again x-ray mineral analysis studies on embryo protein bodies have mostly concentrated upon dicotyledonous plants (Lott 1975, Lott & Vollmer 1979, Lott *et al.* 1978). The monocotyledonous species whose embryo protein bodies have received at least some study with x-ray analysis include Oryza sativa (Tanaka *et al.* 1976, 1977), Avena sativa (Buttrose 1978) and Triticum aestivum (Lott & Spitzer 1980)

The scutellum cells of rice were found to contain P, Mg, and K in the electron dense inclusions of protein bodies (Tanaka *et al.* 1976). The phytin salt was hence a Mg and K salt rather than a Mg and Ca salt. A marked similarity was observed between the elemental composition of the protein bodies in the scutellam cells and aleurone grains from the aleurone layer. Further, in addition to the major elements Mg, K, and P stored as phytin, Mn and Fe have been located in globoid crystals of protein bodies of oats (Buttrose 1978). These results confirmed the chemical analysis reported earlier that Mn was associated with phytin in wheat and oats (Ashton & Williams 1958, Sharma & Dieckert 1975).

Lott & Spitzer (1980)have investigated the mineral distribution in the globoid crystals of wheat embryos. P, Mg, and K were present in all the areas investigated; in most of the embryo regions examined, a few globoid crystals contained Ca and Fe along with P, K and Mg. A well-defined elemental distribution occurred with Mn; it was found only in globoid crystals located in the base and mid-regions of the stele in the radicle.

1.7 Mineral transport to the developing grain

Inorganic plant nutrients may reach the developing grain directly from the soil during the period from pollination to grain maturity and indirectly through translocation of previously accumulated plant nutrients from various plant parts to the developing ear. The latter supply mechanism is known as nutrient redistribution.

1.7.1 Accumulation from soil

The mechanism of uptake of mineral ions from the soil into the root cells has been discussed in detail (Anderson 1973, Clarkson, 1974, Wardlaw & Passioura 1976). This involves apoplastic and symplastic movement leading to release into the xylem vessels followed by the upward movement of ions in the xylem vessels. Relatively large amounts of dissolved ions are present in the xylem and their rates of upward movement were found to be related to the rate of transpiration (Kramer 1969). The composition and concentration of xylem sap varies with the species, the season and the time of the day. The ionic composition and concentration of rooting medium also exerts a profound effect on xylem sap.

Not all minerals are transported in xylem as inorganic ions. For example, most of the nitrogen is transported in the form of amides and amino acids. In addition the movement of certain ions through the xylem may be restricted by their tendency to precipitate. Fe may be precipitated as ferric phosphate and deficiency of Fe may occur even though adequate quantities are being absorbed by the roots.

The mechanism of absorption by cells via active transport or passive diffusion has been discussed elsewhere and will not be discussed here.

1.7.2 Redistribution

Redistribution of nutrients, including inorganic ions, occurs throughout the growth cycle of a plant but is particularly significant during the growth and maturation of the grain. Mason & Maskell (1931) studied the movement of carbohydrate and other solutes in the phloem of cotton plants and introduced the concept of sources and sinks. Sinks are now mainly taken as growing regions of the plant e.g. meristems, young leaves and developing storage organs towards which materials move from sources of supply elsewhere in the plant.

The redistribution of minerals during development has been studied in a number of important crop plants such as oats and soybeans (Williams 1955, Ohlrogge 1963). Williams (1955) reported that certain cereals, including oat plants, accumulated 90 per cent of their final N and P contents at a stage when they had reached only 25 per cent of their final dry weight. Thus it seems clear that some redistribution is needed to allow completion of the plants' development.

Mineral ions may also be redistributed to the developing fruit from senescing tissue (Gregory 1937, Noodén & Leopold 1978). Removal of the young fruit, apparent sinks, prevented or delayed senescence in many plants (Lindoo & Noodén 1976). Whether the nutrient drain causes senescence or the efflux was a consequence of senescence is not clear (Noodén & Leopold 1978).

The extent of retranslocation of specific mineral nutrients during both plant development and senescence depends largely on the relative mobilities (See 1.8) of the ions in the vascular tissues i.e. the xylem and phloem.

1.8 Ion mobilities

Storage organs and in particular seeds, are heavily dependent, if not entirely so, on outside sources of nutrients, since they are relatively ineffective in capturing water through transpirational activity. Hence, it appears that phloem transport is the prominent feature in the nutrition of seeds (Wiersum 1966, Milthorpe & Moorby 1969, Zimmerman 1969).

Earlier investigations indicated that there were differences in the phloem mobility of different inorganic substances (Mason & Maskell 1931). With the help of radioisotopes Bukovac & Wittwer (1957) systematically studied the mobility of mineral elements in the phloem of bean plants. They classified them into three groups of decreasing mobilities as shown in the table below:-

Table - 1.2 Mobility of mineral elements in the phloem (Bukovac & Wittwer 1957)

Mobile	Intermediate	Immobile
K	Fe	Li
Rb	Mn	Ca
Na	Zn	Sr
Mg	Cu	Ba
P	Mo	B
S		
Cl		

Similarly in studies relating to phloem and xylem transport of minerals to developing legume fruit, it was reported that P, K, Mg, Zn; were transported mainly in the phloem, and Ca and Na mainly in the xylem (Pate & Hocking 1978, Hocking *et al.* 1978). Mn proved to be fully mobile in xylem but only sparingly mobile in phloem (Hocking *et al.* 1977).

The rapid mobility of N, P, K has several consequences. Plants which have accumulated excess concentrations in their leaves may continue to grow unchecked even when they have no external supply of these nutrients. Moreover, deficiencies do not develop until the total amount of nutrient in the plant as a whole becomes inadequate. The deficiency symptoms then become apparent first in older leaves which supply the young developing parts of the plant via the phloem.

The characteristics of nutrient deficiency for mobile nutrients stand in direct contrast with those for immobile nutrients such as Ca. Once deposited in plant leaves, Ca becomes virtually immobile. As a result, plant organs can only grow if they receive a continuous supply of Ca from the external medium or from the transpiration stream (Haynes & Robbins 1948, Harris 1949, Wiersum 1966). The development of Ca deficiency symptoms are first seen in young leaves even though the older leaves may contain excess Ca (Loneragan & Snowball 1969).

The degree of variability in the mobility of some of the 'intermediate mobile' nutrients may be quite high within a species (Loneragan *et al.* 1976) depending upon environmental conditions and upon the stage of plant growth. Mobility of these nutrients also varies strongly with the adequacy of supply of the nutrient itself; mobility is highest at luxury concentrations and lowest at deficient concentration (Loneragan *et al.*1976, Single 1958).

1.9 *In vitro* embryo culture

The growth of plant embryos in culture has been the subject of much investigation since the beginning of the last century. The great interest in this field is due partly to the opportunity that is provided for observing the development of adult structures from cells that are poorly differentiated, and partly to the possibilities it offers of producing seedlings directly from embryos. This is particularly pertinent to those seedlings derived from many hybrid crosses which can not develop during *in vivo* seed maturation because of incompatibility between the embryo and endosperm.

1.9.1 Immature embryo culture

Unlike the mature embryos, the culture of isolated immature embryos proved not to be an easy task because of their critical nutritional requirements. The key for the successful culture of pro-embryos was the discovery that they required a more complex growth medium than that required for mature embryos. In addition the optimum growing medium for *in vitro* culture was one which imitated closely the composition of the endosperm or the milieu of the embryo sac. This was presumably due to the low synthetic capacity of the embryo at the pro-embryo stage. Thus they require amino acids, carbohydrates, purines, pyrimidines, perhaps vitamins, plant hormones and other essential metabolites present in the embryo sac. As development proceeds the synthetic capacity increases and the embryo becomes less dependent on its environment.

In order to study the complete development of the embryo after fertilization to maturity, it is necessary to excise and culture pro-embryos. White (1932) isolated young heart shaped embryos of Portulaca oleracea and cultured them apparently successfully on media containing mineral salts, glucose and fibrin digest.

Similarly La Rue (1936) cultured small embryos (0.5 mm) from both dicotyledonous and monocotyledonous plants on media containing yeast extract, inorganic salts with sugar and indoleacetic acid. Seedlings capable of living in the soil were obtained. However, it was not possible to culture embryos less than 0.3 mm long.

To lower this threshold, overcome precocious germination and increase the survival of the immature embryos, the effects of additions of natural plant extracts, protein preparations and the role of high osmotic pressure have been investigated. The cessation of normal embryo development accompanied by germination at a less than normal embryo size is termed precocious germination.

Several natural plant extracts e.g. coconut milk, water extracts of dates and bananas, wheat gluten hydrolysate and tomato juice have been used (Kent & Brink 1947, van Overbeek *et al.* 1941, 1942). Although coconut milk was used more frequently, it did not always reduce the incidence of precocious germination in grass embryos (Ziebur & Brink 1951, Haagen-Smit 1945, Norstog 1961). In addition the precise chemical nature of the stimulating agents in the plant extracts was not clear and hence media with a more defined composition were required (Norstog & Smith 1963).

The addition of casein hydrolysate (1 per cent) to basal media was observed to cause a marked enhancement of embryo growth and an inhibition of precocious germination (Ziebur *et al.* 1950, Mauney 1961). The growth promoting effect of this protein preparation was probably due to its high osmotic pressure rather than to its nutritional properties.

1.9.2 High osmotic concentration

Since the osmolarity of the liquid endosperm constantly bathing the embryo is high (Mauney 1961, Smith 1973), a favourable osmotic concentration in the culture medium, besides preventing a possible osmotic shock to the embryo, also inhibits the cell elongation usually observed during precocious germination. The cells are switched from a state of cell elongation to cell division (Hannig 1904).

Good evidence is available for a direct relationship between osmolarity of the medium and growth of small embryos (Kent & Brink 1947, Ziebur *et al.* 1950). When studying the effects of casein hydrolysate on growth of immature barley embryos; the effects could not be duplicated solely with a mixture of amino acids and inorganic phosphate and sodium chloride, the additional components of casein hydrolysate were also required. It was assumed that this was purely an osmotic effect.

The osmotic concentration of culture media has been artificially increased by using mannitol or sucrose. Although precocious germination was prevented by addition of casein hydrolysate during culture of larger embryos, the continuation of growth of smaller embryos was only achieved by addition of 12.5 per cent sucrose (Ziebur *et al.* 1950, Ziebur & Brink 1951). Norstog (1961) used a similar sucrose concentration to extend the period of barley embryo growth and later Norstog & Smith (1963) found 9 per cent to be an optimum sucrose concentration in their defined medium. In general, older and larger embryos can grow in sucrose concentrations lower than those required to maintain growth in culture of smaller embryos (Paris *et al.* 1953). The beneficial effects of such high sucrose concentrations are attributed in part to the osmotic pressure as well as to its nutritional properties. The high osmotic value in the medium has been suggested to allow for an effective flow of metabolites into the growing cells. However, no specific data are available to support this hypothesis.

1.9.3 Other aspects of the culture medium

The role of the culture medium is to supply the nutrient requirements during embryogenesis. Nutrient requirements will presumably vary throughout the developmental time scale and so the ideal culture medium is one which not only contains the right nutrients but also varies in composition in a way that parallels that supplied by the parent plant.

1.9.3.1 Carbohydrate nutrition

Sucrose seems to be the optimal carbon source for most embryos grown in culture. It was found to give the best results with Datura (van Overbeek *et al.*1944) Capsella (Rijven 1952) and cotton embryos (Mauney 1961). Similarly, Cockerline (1961) investigated the effect of a wide variety of disaccharides, hexoses and pentoses as well as glycolytic intermediates on the growth of barley embryos in culture and concluded that sucrose was the optimal carbon source.

The role of sucrose as the sole carbohydrate source in culture media is not clear. A higher permeability of the plasma membrane of scutellum cells to monosaccharides rather than disaccharides was reported for isolated immature embryos (Cameron-Mills & Duffus 1979). However, the ability of very young embryos to utilise sucrose is supported by the appearance of enzymes involved in sucrose cleavage early in embryo development (Duffus & Rosie 1975). During studies on the culture of barley (Norstog 1967) and cotton embryo (Mauney *et al.* 1967), malic acid was found to have a beneficial effect both as a carbon source and as a buffer.

1.9.3.2 Mineral nutrition

White (1943) recommended a mineral medium containing the major elements N, P, S, K, Ca, Mg and trace elements Fe, Mn, Zn, B and I. In the pursuit to improve the viability of small embryos, the medium has been modified not only by addition of organic compounds (sec. 1.9.1), but also in its inorganic component.

Thus when the phosphate concentration in White's medium was increased almost fifty fold, barley embryo growth was enhanced (Norstog & Smith 1963). However, several other modifications were also made at the same time e.g. additions of glutamine and alanine and adjustments of pH; the sole effect of increase in phosphate concentration alone was not clear. Later, Norstog (1967) increased K salt concentrations 5 - 10 times, keeping the concentrations of all the other constituents constant, and observed marked improvement in the viability of barley embryo 0.2 - 0.4 mm long. Further, when the Ca concentration, in White's nutrient medium with high-phosphate modification of Skoog and Tsui (1948), was increased 5 times, a substantial increase in the growth rate of developing cotton embryos was observed (Mauney 1961). A further slight increase in growth rate resulted when all salts were raised to 5 times the level recommended in Skoog and Tsui's modification of White's medium.

The mineral composition of the different media used in embryo cultures varies mainly in the concentrations of the major and trace elements as suggested in White's medium. However, Cu, Co, Mo ions have not been included in the trace element component (Norstog & Smith 1963, Norstog 1973, Kasha & Kao 1970).

The type of N source in culture media has received attention due to the well established role of N as a raw material for protein synthesis. Both inorganic and organic forms of nitrogen have been recommended for successful culture. Ammonium nitrate was found to be significantly superior to potassium and sodium nitrate (Matsubara 1964). Ammonium malate was the preferred nitrogen source in barley embryo culture and when the concentration was increased by 10 fold in Norstog medium II as compared to medium I, a marked improvement in growth was observed (Cameron-Mills & Duffus 1977).

The main nitrogen source in the media is however supplemented with low concentrations of a complex of amino acids. A comparative study on the utilization of glutamine and asparagine (400 mgl^{-1}) by embryos of twelve species of angio-sperms, including Hordeum vulgare (embryo length 0.6 mm) showed that in all cases glutamine had a greater growth-promoting effect (Rijven 1956). Furthermore, the addition of glutamine to medium containing casein hydrolysate induced embryo growth and increased the growth potential of small embryos (Cameron-Mills & Duffus 1977). The central role played by glutamine in relation to carbohydrate and nitrogen metabolism could explain this effect.

1.9.3.3. pH of media

This is an important property of the medium and cultured embryos show poor growth at unsuitable pH. Rijven (1952) ascertained that the ovular sap in Capsella was about pH6.0, and cultured embryos responded equally well to media ranging from pH5.4 to 7.5. Media adjusted to pH4.0 caused death in around one fifth of the embryos. Ziebur *et al.* (1950) found that barley embryos less than 0.55mm long failed to grow at pH5.6 and Cockerline (1961) observed that embryos 0.3mm long failed to grow at pH5.7 in a number of media. Further experiments showed that the successful pH range for barley embryo culture was very narrow, between 4.9 and 5.2. Beyond pH5.2 very little differentiation occurred, but an increase in cell elongation was observed (Norstog & Smith 1963). However, whether the pH manipulations were carried out at constant ionic strength is not clear.

1.9.3.4 Use of Agar in culture media

Most successful embryo cultures appear to have been carried out on agar surfaces, although some limited success has been reported in liquid cultures.

The concentration of agar appears to be critical for root growth. Shoot growth on the other hand was not affected by changes in concentration (Khan & Randolph 1960). Liquid culture media where all nutrients were present at optimum concentrations, even when aerated could not support growth of cotton embryos (Mauney 1961). In contrast, embryos placed in surface moisture on an agar medium developed rapidly. Further, barley embryos, when submerged in a culture medium containing agar, did not grow (Norstog 1961). Hence it appears that embryos grow best on surfaces which presumably most closely mimic their *in vivo* environment, that of a semi-solid endosperm. The failure of embryos to grow in liquid media may be due to the limited availability of oxygen due to restricted diffusion. On the other hand, the head space above the solid medium would provide adequate oxygen to the embryo cells.

Aim of the Work

Previous work in this laboratory (Cameron-Mills & Duffus 1977) has established that developing barley embryos are highly sensitive to changes in the growth environment. For example, changes in the sucrose and amino acid supply can result in abnormalities of embryo structure coupled with variation in the growth rate and subsequent ability to germinate. The aim of the present work is to examine the effect of variation in the supply of selected essential mineral ions on the growth and development of barley embryos. Although it is clear that many of the processes concerned in the overall development of plants require the presence of a range of mineral elements, little is known of their role in embryo development.

2.0 Materials and Methods

2.1 Glass double distilled water

All glass double distilled water was used throughout the present investigation i.e. for washing, in dilutions and in samples. Samples of the water were analysed periodically for mineral element content. None of the elements studied was detected.

2.2 Plant material

The two row barley Hordeum vulgare L var distichum cv Midas, Julia, Zephyr and Hassan was grown under greenhouse conditions and daylight supplemented with 'Phillips HLRG 400 watts Giant Edison Screw' mercury vapour lamps to give a 20h day. The average greenhouse temperature was maintained at 18C.

Plants were grown in 17.8 cm polypropylene pots at a final density of six plants per pots. Ten seeds (undressed) were sown in each pot and seedlings thinned. The fertilizer, ICI Solufeed Standard (23 per cent nitrogen, 19.5 per cent phosphoric acid and 16 per cent potash) was applied once a week throughout plant growth.

Ears were harvested at various stages of development and when not used immediately were kept at -18C until required. Measurements were completed within 6 months of harvesting. Each ear had between 24-30 embryos. Embryos sampled were always from grains in the middle of the ears.

2.3 Ageing

The date of fertilization (anthesis) was determined by the method of Merritt and Walker (1969) in which the anthers were dissected out and tested for the release of pollen. Another method used was that described by Baxter (1972) in which grain age was related to various morphological markers. Under the growth conditions used the grain reached maturity by around 60 days after fertilization.

2.4 Measurements of embryo size

Embryos were placed on a clean sterilized petri dish immediately after dissection from the grain and the length and width measured using a Will Zoom Stereo-microscope equipped with an ocular micrometer. Embryo size was measured in mm^2 as the product of length and width.

2.5 Measurement of embryo and endosperm dry weight

Embryos and endosperms were placed in washed, preweighed platinum crucibles and dried at 80C until constant weight was reached. For all embryo and endosperm ages 48h and 72h respectively proved to be sufficient. Crucibles were allowed to cool in a desiccator before reweighing.

2.6 Essential washing procedure used for mineral element analysis

2.6.1 Platinum crucibles

Platinum crucibles were cleaned by boiling in concentrated hydrochloric acid for 15 minutes followed by rinsing several times with double distilled water.

2.6.2 Glassware and polyethylene containers

All glassware and polyethylene containers used were steeped in Decon 90 for 24 hours, washed and rinsed with double distilled water. They were then immersed in dilute nitric acid (N) for a minimum of 24 h or until required. Before use they were rinsed 4 times with double distilled water.

2.7 Preparation of material by wet ashing for atomic absorption spectroscopy (AAS)

For these experiments grain aged between 12 and 60 days after anthesis was used. Each age sample constituted 10 embryos or endosperms sampled from the middle of 3 ears, harvested randomly from the pots.

2.7.1 Embryos

The embryos were dissected out with the use of EMscope forceps No.3, after the outer layers palea, lemma and pericarp had been removed from the intact grain.

The size of the embryos was determined using microscopy as described in sec. 2.4. The material was dried in platinum crucibles and dry weights obtained as described in sec. 2.5. A minimum of 10 embryos was sampled from the central position of 3 ears for all ages except for those younger than 15 days, when 20 embryos were required.

The dried embryos were transferred to washed boiling tubes (sec. 2.6.2) with a platinum spatula and 2 cm^3 of 50 per cent (7.9M) Aristar nitric acid in glass double distilled water were added (Culver 1975). The tubes were transferred to a cold oil bath (Techne) and heated for 40 min during which period the nitric acid evaporated leaving a yellow ash. The final temperature reached was 140C. The walls of the tubes were rinsed three times with double distilled water and the cooled acid hydrolysate made up to a final volume of $2cm^3$ with double distilled water. The solution was transferred immediately to polyethylene storage bottles. A tube containing $2cm^3$ of nitric acid (50 per cent) was included in every digestion as a reagent blank.

2.7.2 Endosperms

Endosperms (including the aleurone layer) were similarly dissected by hand and sampled like the embryos. Endosperms were wet ashed as above, but with $3cm^3$ of 50 per cent nitric acid and the samples were made up to a final volume of $10cm^3$.

2.8 Recovery of (exogenous) metal ions and reproducibility of results

Mineral element recoveries were estimated by assaying embryo and endosperm digests to which known amounts of mineral ions had been added. Embryos and endosperms at 3 different stages of development were used.

In each case, the mineral elements were added at 3 different concentration levels. The concentrations added were in the same order as those expected in the original sample. Each sample contained 10 embryos and 10 endosperms.

For each of the 3 ages (20, 30, 40 days) of embryos and endosperms used, the following mineral ion additions were made:-

Table 2.1: Concentration of mineral elements added (mgl^{-1}) to 10 embryos in $2cm^3$ extract.

	Concentrations of mineral elements added (mgl^{-1})					
	K	Mg	Ca	Fe	Cu	Mn
Embryos +	20.0	2.5	1.0	0.30	0.20	0.10
Embryos +	40.0	5.0	3.0	0.60	0.50	0.20
Embryos +	60.0	10.0	5.0	1.20	1.00	0.30

Table 2.2: Concentrations of mineral elements added (mgl^{-1}) to 10 endosperms in $10cm^{-3}$ extract

	Concentration of mineral element added (mgl^{-1})				
		K	Mg	Ca	Mn
Endosperms	+	20.0	2.5	1.0	0.10
Endosperms	+	40.0	5.0	3.0	0.20
Endosperms	+	60.0	10.0	5.0	0.30

Samples were triplicated for every age and concentration. Stock solutions of standards to be added were made such that final nitric acid concentration remained 7.9M The ashing was completed as desribed in sec. 2.7 and the cooled hydrolysate made up to a final volume of $2cm^3$.

2.9 Mineral element analysis

All elements were analysed using AAS. The major elements K, Ca, Mg were atomized using an air-acetylene flame. Double distilled water was aspirated alternately between samples to compensate for noise signal and instrumental zeroing. A complete standard-calibration curve was prepared from a series of single-element standard solutions aspirated immediately before and after aspiration of samples. The trace elements Cu, Fe and Mn were analysed by flameless AAS, using the carbon rod atomizer (Varian CRA 63). An Oxford 3000 series Laboratory chart recorder was used to record the atomizing peaks.

The conditions used with the atomic absorption spectrophotometer Varian AA5 with CRA 63 are summarised in the table 2.3. Double distilled water was analysed at the start of each run to test for any mineral ion contamination. A series of standards in double distilled water was prepared for each element analysed and standards were run immediately before and after analysis of samples. Commercially available (BDH) nitrate stock solutions ($1.0 g l^{-1}$) specifically for AAS were used. Single-element standard solutions of 10 $mg l^{-1}$ were made every 3 months. Working standards as shown in table 2.3 were prepared from 10 $mg l^{-1}$ solutions on the day required for analysis. The heights of the absorption peaks obtained for each standard were plotted on a calibration graph and the concentration in the sample determined by interpolation. Each standard and sample point was determined in triplicate.

Table 2.3: Atomic absorption spectrophotometer (model AA5) operating conditions. Instrumental parameters and standard ranges used in analysis of mineral elements using flame and flameless atomizers.

Mineral element	Standard ranges mgl^{-1}	Cathode ray lamp current mA	Wavelength nm	Spectral Band width nm	Flame Air-acetylene pressure $x10^5$ Pa	Flameless CRA 63 parameters	
						Ash setting	Atomize setting
K	1.0 - 6.0	6.0	769.9	0.5	0.7		
Mg	0.05- 0.4	2.0	285.2	0.2	0.7		
Ca	0.5 - 3.0	5.0	422.7	0.2	0.7		
Cu	0.01-0.12	6.0	324.8	0.2		*6.5/20	*8.0/2.5
Fe	0.01-0.15	9.0	248.4	0.2		7.2/20	8.3/2.3
Mn	0.01-0.04	7.0	279.5	0.2		6.4/15	7.0/2.7

* First figure :- Temperature Ashing and Atomizing setting
Second figure :- Duration in seconds

Oxygen free Nitrogen flow rate for flameless atomization 0.7 x 10^5 Pa.

2.10 Plants grown in sand culture

2.10.1 Materials and washing procedures

Polyethylene pots (23 cm) were washed in Decon 90, rinsed with M nitric acid and several times with glass double distilled water. Polyethylene storage bottles (2l) for stock solutions, aspirators (25l) for nutrient solutions and all glassware were washed as described earlier in sec. 2.6. All pipettes and beakers used for dilution and dispensing were steeped in M nitric acid until required. Washed polypropylene rods were used to tie the plants at later stages of growth.

Double Arches Pit No. 21 washed silica sand was supplied by Messrs. Arnolds Quarries, Leighton Buzzard, (Hewitt 1966). For Mg nutrition studies, the sand was leached 4 times with glass double distilled water before use. In the experiments where only Mn and both Mg and Mn were withdrawn from complete nutrient solution; the following washing procedure was carried out. Sand (98kg) was steeped in cold 2M hydrochloric acid and 1.0 per cent (w/v) oxalic acid in a large container for a period of 8 days. During this period the sand was stirred with polypropylene rods frequently. At the end of the acid treatment, the sand was leached with glass double distilled water several times to remove the free acid. This was achieved when the pH of the leachate became neutral. To ensure complete removal of adsorbed hydrogen ions, the sand was further steeped for 24h in twice the concentration of the major element solution described in table 2.4. Washing was continued until there was no further change in the pH of the solution after 24h in contact with the sand.

2.10.2 Plant culture

Barley cv. Midas was grown under greenhouse conditions similar to those described in Sec. 2.2. Plants (6 per pot) were grown in twelve 23 cm washed polyethylene pots containing 7 kg of water-washed or acid-washed silica sand per pot.

When the shoots were 2.5 cm above the surface, all pots were given the complete nutrient solutions up to anthesis. For the Mg nutrition studies (Sec. 7.0) plants were grown in the recommended Long Ashton complete nutrient solution with 1.5 mM Mg (Table 2.4 solutuion a). In the experiments concerning Mn (Sec. 8.0), plants were divided into two sets, one set was grown in complete nutrient solution modified with 0.005 mM $MnSO_4 \cdot 4H_2O$ (Table 2.4 solution d); and the other set received complete nutrient solution with 0.002 mM $MnSO_4 \cdot 4H_2O$ (Table 2.4 solution f).

The various treatments used in the investigation of the effects of withdrawal of Mg, Mn and both Mg and Mn are described below. The treatments commenced at anthesis (7-8 weeks after sowing) and were continued up to maturity in all the experiments. Before the withdrawal of the mineral elements the sand in all pots, including controls, was leached with 4 successive one litre applications of double distilled water followed by 2x1L applications of the new nutrient solutions to the respective pots.

Plants were fed 2-5 times per week with the relevant nutrient solution, depending upon the plant size and the weather conditions. During hot periods every application of nutrient solution was alternated with double distilled water, since high transpiration rates led to leaf browning.

Treatments

In all the treatments the supply of Mg and/or Mn to the plant before anthesis was continued after anthesis in control plants.

2.10.2.1 Mg withdrawal

Plants were grown at 3 different levels of applied Mg after anthesis in which the concentration of Mg in the nutrient solutions were 1.5 mM (control level), 0.3 mM and zero (-Mg). Details of the 3 solutions a, b and c are presented in table 2.4. For the zero and 0.3 mM Mg solutions sodium sulphate was used to replace the Mg such that all 3 nutrient solutions had the same final concentration of anions.

2.10.2.2 Mn withdrawal

Plants were grown at 2 different levels of applied Mn after anthesis, in each set. The concentration of Mn in the nutrient solutions of one set were 0.005 mM (control), and zero (-Mn); and in the other set were 0.002mM (control) and zero (-Mn). Details of these solutions d, e, f, g respectively are shown in table 2.4.

2.10.2.3 Mg and Mn withdrawal

Plants were grown at 2 different levels of applied Mg and Mn after anthesis in which the concentration of Mg and Mn in the 2 nutrient solutions were 1.5 mM Mg, 0.005 mM Mn (control); and zero Mg, zero Mn (-Mg-Mn). Details of these solutions a, d and h, are shown in table 2.4.

Table 2.4: Major and trace element composition of Long Ashton standard complete nutrient solution (Hewitt 1966). Details of stock solutions used for preparation of 25l nutrient solution.

SALT	(a) Complete nutrient solution	
	final conc. (mM)	Vol of stock solution used (cm^3)
Major elements		
KNO_3	4.0	50
$Ca(NO_3)_2$	4.0	50
$MgSO_4.7H_2O$	1.5	50
$NaH_2PO_4 2H_2O$	1.33	25
Trace elements		
Fe Citrate.$5H_2O$	0.10	12.5
$MnSO_4.4H_2O$	0.01	2.5
$CuSO_4.5H_2O$	0.001	2.5
$ZnSO_4.7H_2O$	0.001	2.5
H_3BO_3	0.05	2.5
NaCl	0.1	2.5
$NaMoO_4.2H_2O$	0.0005	2.5

Notes on table 2.4

1. Modifications to complete nutrient solution.

Solutions with varying Mg concentration

(b) 0.3mM $MgSO_4.7H_2O$ and 1.2 mM Na_2SO_4.
(c) No $MgSO_4.7H_2O$ and 1.5mM Na_2SO_4.

Solutions with varying Mn concentration

(d) 0.005mM $MnSO_4.4H_2O$
(e) No $MnSO_4.4H_2O$
(f) 0.002mM $MnSO_4.4H_2O$
(g) No $MnSO_4.4H_2O$

Solutions with varying Mg and Mn concentrations

(h) No $MgSO_4.7H_2O$, No $MnSO_4.4H_2O$, and 1.5mM Na_2SO_4

2. Stock solutions

Major elements: 10 x final concentration
Trace elements: 10 x final concentration

3. All chemicals were Analar grade.

2.10.3 Analysis of the nutrient solutions

A sample of the nutrient solutions a, b, c, d, e, f, g and h (table 2.4) was analysed for Mg and Mn concentrations (sec.2.9) before they were supplied to the respective plants, receiving the treatments described above.

The table below (table 2.5) shows the actual concentrations of Mg and Mn supplied to the sand. Removal of Mg and Mn at different concentrations was effective in producing a depleted nutrient solution.

Table 2.5 Theoretical and analysed concentrations (mM) of Mg and Mn in treatment solutions a, b, c, d, e, f, g and h. (see table 2.4)

Description of nutrient solution as in table 2.4	Analysed Concentration mM		Theoretical Concentration mM	
	Mg	Mn	Mg	Mn
a)	1.50	1.0×10^{-3}	1.50	1.0×10^{-2}
b)	3.8×10^{-1}	1.0×10^{-2}	3.0×10^{-1}	1.0×10^{-2}
c)	4.0×10^{-4}	1.0×10^{-2}	0.0	1.0×10^{-2}
d)	1.56	5.0×10^{-3}	1.50	5.0×10^{-3}
e)	1.35	3.0×10^{-4}	1.50	0.0
f)	1.53	2.0×10^{-3}	1.50	2.0×10^{-3}
g)	1.52	3.0×10^{-4}	1.50	0.0
h)	5.0×10^{-4}	2.0×10^{-4}	0.0	0.0

2.10.4 Sampling

2.10.4.1 Mg withdrawal

All ears were tagged at anthesis as described previously. For each treatment a total of 24 plants was used. Ears were harvested 20, 30, 40 and 50 days after the start of the treatments. On each of the 4 sampling days each plant had ears which varied in developmental age, and 3-4 ears of each age were sampled.

2.10.4.2 Mn withdrawal

All ears in the 24 plants used were tagged. In this case ears that anthesed 15 days after the main ears, were sampled throughout development. 15, 22, 30 and 40 day old ears were harvested for all the experiments.

2.10.4.3 Mg and Mn withdrawal

Similarly, ears that anthesed 15 days after main ears were sampled. 15, 22, 30 and 40 day old ears were harvested from 24 plants.

Ears were stored at -18C until required for analysis. Analysis was completed within 6 months of harvesting.

2.10.5 Estimation of chlorophyll

The seeds from the control plants (1.5mM Mg) and those grown in the absence of Mg were harvested at maturity. A random sample of 12 seeds from each was sown in 4 pots (6 seeds per pot) containing washed sand (sec. 2.10.1). On shoot emergence the seedlings were watered with glass double distilled water and no further mineral nutrients were supplied during seedling growth. The pots were watered once a week. The growth of the seedlings was monitored and harvested at the 4 leaf stage. Seedlings were kept at -18C until required.

Samples of 1st, 2nd, and 3rd leaves were randomly selected from the twelve seedlings of each of the two treatments. To express the results on a dry weight basis each leaf was split down the middle and weighed quickly. Of the two halves one was dried to constant weight at 80C and the dry weight determined; the other half was used for the estimation of chlorophyll. The leaves were homogenized using an all-glass hand-held Potter-type homogeniser and the chlorophyll extracted in 5 cm^3 of 80 per cent acetone. The homogenate was filtered and chlorophyll estimated using the method of Arnon (1949). Absorbances were read at 663nm and 645nm.

2.10.6 Total N content of endosperms

N was determined by micro-kjeldahl digestion of dried endosperms (10 per sample). Three ages each (25, 30 and 40 day old) of -Mg-Mn and control plants were analysed. The results were expressed as N content per g dry weight.

2.11 *In vitro* culture of embryos

2.11.1 Preparation and composition of culture media

2.11.1.1 Culture media for embryos grown in petri dishes

Stock solutions for the various components of the medium were prepared as shown in Table 2.6. These were used as described below in the preparation of $100cm^3$ of culture media: $50cm^3$ major minerals solution and $10cm^3$ trace elements solution were added to 0.6g Difco Purified agar or Bactoagar. The pH was adjusted to 4.9 with potassium hydroxide and the mineral-agar mixture autoclaved at 0.1 MPa for 20 min.

All subsequent manipulations were carried out in a 'Microflow' laminar flow cabinet. When the mineral-agar mixture had cooled to 60C, the following were added together: Seitz filtered vitamins mixture ($1cm^3$), amino acid mixture ($10cm^3$), 6.4mM malic acid at pH5 adjusted with ammonium hydroxide ($5cm^3$) and sucrose solution ($10cm^3$). The sucrose solution was autoclaved separately. After making up to 100 cm^3 with warm sterile double distilled water, the solution was poured into disposable sterile petri dishes (6cm diameter). Two sets of media were made up, one with a final concentration of 0.1M (3.42 per cent w/v) sucrose and one with a final concentration of 0.2M (6.84 per cent w/v) sucrose. About $25cm^3$ of medium were poured when warm into each dish and allowed to set. The lids were sealed with two way stretch parafilm and the dishes stored at 4C.

2.11.1.2 Culture media for embryo germination in test tubes

$5cm^3$ of medium prepared as above was poured into each sterile disposable test-tube (10.0 x 1.6cm). The final concentration of sucrose was 0.1M.

The complete composition of the medium used for barley embryo culture *in vitro* is shown in table 2.6. This is essentially Norstog's Medium II (Norstog 1973). The pre-germination medium (petri-dishes) contained 0.2M sucrose whereas the post-germination medium contained 0.1M sucrose.

Table 2.6:- Norstog Barley Medium II

Component	Final Concentration	Component	Final Concentration
Major Elements	gl^{-1}medium	Vitamins	mgl^{-1}medium
KH_2PO_4	0.91	(meso) Inositol	50.0
KCl	0.75	Thiamine-HCl	0.25
$MgSO_4.7H_2O$	0.74	Ca-pantothenate	0.25
$CaCl_2.2H_2O$	0.74	Pyridoxine -HCl	0.25
Trace Elements	mgl^{-1}medium	Amino acids	gl^{-1}medium
$MnSO_4.4H_2O$	3.0	L-Glutamine	0.4
H_3BO_3	0.5	L-Alanine	0.05
$ZnSO_4.7H_2O$	0.5	L-Cysteine	0.02
$CoCl_2.6H_2O$	0.025	L-Arginine	0.01
$CuSO_4.5H_2O$	0.025	L-Leucine	0.01
$Na_2MoO_4.2H_2O$	0.025	L-Phenylalanine	0.01
Fe-citrate	10.0	L-Tyrosine	0.01
		Other additions	
Sucrose	34.2	Malic acid	1.0
or	68.4	Difco purified Agar	6.0
		Difco Bactoagar	6.0

cont. of table 2.6

N.B. Concentration of stock solutions used.

	Multiple of final concentrations
Major elements	2
Trace elements	10
Vitamins	100
Amino acids	10
Malic acid with pH adjusted to 5.0	20
Sucrose	10

2.11.2 Collection of plant material

The two row barley Hordeum vulgare L. var distichum cv Midas was grown in a greenhouse under conditions described in sec. 2.2. The first 6 tillers of any plant were tagged at anthesis and the ears harvested 10-15 days later. Embryos 10 days after anthesis were 0.45mm long. The grains from the central portion of each ear were dehusked by hand, sterilized in 5 per cent (v/v) sodium hypochlorite for 15 min and rinsed thoroughly in sterile glass double distilled water.

2.11.3 Excision of embryos

All subsequent operations were carried out in a 'Micro-flow' laminar flow cabinet. Individual sterilized grains were placed in a drop of sterile 6 per cent (w/v) sucrose on a sterile petri dish to prevent any desiccation and osmotic shock during the subsequent manipulations. Using a dissecting microscope, illuminated from both top and bottom to facilitate location of the embryo, and very fine tweezers (EMscope No. 3 and No. 4), the embryos were carefully teased apart from the beak like structure to which they

are attached. The embryos were scooped out in a droplet of sterile 6 per cent (w/v) sucrose solution and transferred to the culture medium. The size of the embryos was determined with an ocular micrometer fitted in the dissecting microscope. The tweezers were autoclaved before the start of every culturing session, and frequently flame sterilized with absolute ethanol and rinsed in sterile distilled water during the operation.

2.11.4 Embryo growth

Embryos were placed carefully on the medium such that the scutellum was directly in contact with its surface. The bigger embryos ($0.38mm^2$), where the coleoptile indentation was visible, were placed 'face-up' so that the coleoptile, shoot primordium and coleorhiza would be exposed to air.

Initially the embryos were grown in petri dishes and incubated in the dark at 20C. Usually 8 to 10 embryos were grown in one dish. During this period, embryo growth was monitored using a Will Zoom Stereo-microscope equipped with an ocular micrometer. Growth was measured by increase in embryo dimensions (l x w). When embryos had reached a size of $9.0mm^2$ they were transferred to test tubes and placed in an illuminated growth cabinet maintained at 20C with an 18h day and 8,000 lx illumination.

The use of two different growth vessels was due to the need for convenient microscopic measurements during early development. This was facilitated by growth in petri dishes. Obviously germination and subsequent growth could not be studied under these conditions and so transfer to test tubes was required.

2.11.5 Photographic procedure

Embryos cultured *in vitro* were removed from petri dishes at the required stages of development and photographed immediately using a 35mm single lens reflex camera (Soligor TM) adapted for dissecting microscope. A 5X lens was used as the eyepiece fitted in the adaptor between the microscope and camera. A Kodak film (PX135-20) with a speed of ASA 125 was used. Similarly embryos *in vivo* of corresponding sizes were dissected from grains obtained from plants grown in the greenhouse and photographed.

2.11.6 Growth on medium at varying sucrose concentrations

Two sets of petri dishes containing culture media at varying sucrose concentrations of 0.1M and 0.2M were prepared as described in sec. 2.11.1.1. Embryos 0.23 and 0.38mm^2 in size at excision were cultured as before (sec. 2.11.4). Growth was monitored as increase in embryo size (sec.2.4).

2.11.7 Growth on medium containing no added Mg

The culture medium was prepared as in sec. 2.11.1.1, but $MgSO_4.7H_2O$ was not added to the major element fraction. On sterilization the medium was poured into petri dishes. Embryos were dissected, placed on the medium as before (sec.2.11.4) and cultured in the dark at 20C. Embryo growth was monitored every second day of culture by size measurements (sec. 2.4). Control embryos were grown on medium containing the complete complement of major elements i.e. including $0.74gl^{-1}$ $MgSO_4.7H_2O$.

2.12. Electron Microscopy.

2.12.1 Embryos

Embryos at two different developmental stages were dissected out from freshly harvested grains under a fixative containing 2.5 per cent glutaraldehyde in 0.025 M phosphate buffer pH7.2.Embryos from *in vitro* studies were removed from the culture media and placed in the fixative. The embryos were kept in this fixative at 4C for 20h, post-fixed at room temperature in 2 per cent (w/v) osmium tetroxide for 2h, dehydrated in an acetone series and embedded in an epon/araldite resin. Thin sections, cut on an LKB ultramicrotome, were stained in uranyl acetate and lead citrate and examined in an AEI EM802 electron microscope at 60kV. The embryos illustrated in Plates 11.1-11.3 were embedded in Spurr's resin (Spurr 1969).

2.12.2 Leaves

Transverse slices 1mm thick were cut from the leaves under the fixative and prepared for transmission electron microscopy in exactly the same way as the embryos.

3.0 Tissue preparation and mineral element determinations

3.1 Introduction

The determination of mineral elements in organic materials requires its destruction by either wet or dry oxidation, in order to remove interfering matrix components.

Gorsuch (1959) has assessed exhaustively the relative merits of wet and dry oxidation. The advantages of wet oxidation include a liquid system which reduces the chance of sample loss by volatilization; rapidity of sample oxidation; and relatively simple apparatus. The main disadvantage is the risk of increased blank value by the addition of reagents. On the other hand the main advantages of dry ashing are the absence of added reagents, and the ease with which samples can be handled. However, the disadvantages include: a lack of knowledge of the nature of the interaction between sample constituents, the elements and the material of the receptacle due to the high temperatures required (400-800C); losses due to volatilization; the need for expensive digestion equipment and a relatively long period required for oxidation. Generally, no one method is suitable for any combination of elements and organic material.

The commonest methods for wet acid digestion are oxidation with mixtures of either: nitric and sulphuric acids; nitric, sulphuric and perchloric acids; nitric and perchloric acids; or nitric acid alone. The nitric and sulphuric acid digestion is slower than the others and the presence of sulphuric acid gives rise to insoluble salts especially those with Ca. The recovery of lead (Pb) in cocoa was found to be very variable when a mixture of nitric, perchloric and sulphuric acids were used (Gorsuch 1959).

Further, extreme care has to be exercised when using perchloric acid due to the possibility of explosion.

Nitric acid by itself has been less frequently used for ashing. Nitric acid (M) was used in the digestion of human liver tissue in the analysis of Zn, Cu and Cd (Evenson & Anderson 1975). Garcia *et al.*(1972) also described a rapid wet-ashing procedure for determination of mineral constituents in corn and wheat embryos using concentrated nitric acid. The elements were finally taken up in hydrochloric acid. The ashed residue which dissolved to give a clear solution with no requirement for filtration, was analysed for eight different elements by flame AAS. The reproducibility of all the elements in seven replicate samples was observed to be good. However, no experiments were done to establish the per cent recovery of the elements after this acid digestion.

For a number of reasons, AAS has replaced chemical methods for analysis of metal ions. Early investigations into the application of AAS in the analysis of plant material for Zn, Mg, Cu and Fe were made by David (1958) who commented favourably on the accuracy, rapidity and sensitivity of the technique. In addition, preparations of samples for AAS are generally simple since the technique is very specific and chemical separations are rarely necessary. Hence this method has an advantage over others in that the number of stages of preparation, as well as the reagents used, can be cut down markedly. Zief and Horvath (1976) reported that contamination from particulates in air, impurities in reagents and leaching from containers was a major source of inaccuracy in metal ion analysis and suggested that the number of stages for preparation and analysis of samples should be minimised.

Atomic absorption measurements can be subject to a number of interferences : matrix effects influencing the amount of sample reaching the flame; chemical interferences affecting the number of atoms formed in the flame; and non-atomic absorption by undissociated molecules in the flame. Matrix effects can be tested by adding combinations of salts, that are known to be present in the tissue, to the metal ion standards in aqueous solution. The proportion of sample aspirated that reaches the flame is dependent on the viscosity, surface tension and density of the sample solution. To minimise differences between sample and standard, it is necessary that the physical properties of both are matched as closely as possible. This interference is not present when flame-less methods are employed for atomisation.

The most common form of chemical interference is the formation in the flame of compounds such as calcium phosphate and potassium fluorotantalate. Such interferents form compounds which are not completely dissociated at the temperature of the flame. The extent to which chemical interferences affect the absorbance is dependent on the type of flame used. Ca is one of the most susceptible elements to interference in the air-acetylene flame.

While Na, K and Mg enhance the absorption due to Ca by around 5 - 10 per cent, other interferants (e.g. Be, Al, Fe, SO_4 , PO_4 and SiO_3) depress the absorption of Ca (Adams and Passmore 1966, Sastri *et al* 1969). However, about 20 - 50 times the concentration of interferent has to be present to cause a significant effect (Parker 1972). Few interferences have been found to be significant in the determination of Mg in the air-acetylene flame (Ramakrishna *et al* 1968). K also appears to be relatively free of interferents. Most of these interferences in the determination of Ca can be eliminated by addition of lanthanum or strontium (Pawluk 1967, Parker 1972). For example, phosphate interference in the determination of Ca in triticale was overcome by this procedure (Lorenz *et al* 1974).

Non-atomic absorption generally increases at high molecular concentrations and lower wavelengths. This type of interference can frequently be overcome either by operating at higher wavelength or by using a higher temperature flame, to improve the dissociation of molecules. In some cases neither of these methods may be possible, and non-atomic absorption must then be measured using the hydrogen continuum lamp.

During the present study, a rapid method for decomposition of embryo and endosperm of barley grain was required and which had no incidental losses of elements. An additional requirement was that the ashed sample would easily give a clear solution directly compatible with AAS. Chemical compositions of biological ashes vary widely and hence the behaviour of various types of samples in ashing processes differ. Both corn and wheat embryos, where the major inorganic constituents are P, K and Mg, wet-ash easily with concentrated nitric acid at low termperatures (Garcia *et al*.1972). Barley embryos have a similar composition (Duffus & Rosie 1976 a) and nitric acid was therefore selected for wet ashing. This method has the further advantage of reducing contamination from reagents since nitric acid, the only reagent, can be efficiently purified. Complete solublisation of the material was achieved at the end of the digestion.

In an earlier study on the changes in trace element composition of developing barley grains (Duffus and Rosie 1976 b), plant material was dried at 450C and elements extracted in 6N hydrochloric acid. The filtered solution was analysed for Zn and Cu in addition to Mn and Fe. Various reports documented by Gorsuch (1959) list the effects of dry and wet oxidations on the recovery of Zn and Cu. Overall it appears that in both cases, retention on the basins would introduce an error during dry oxidation. No obvious problems however were encountered in the recovery of these elements during wet oxidation.

Filtration of the solution which could introduce contamination and the large sample size were two other points against using the above procedure. Similarly for the analysis of major elements, sulphuric acid in the presence of which insoluble calcium sulphate is formed was used during ashing (Duffus and Rosie 1976 a). Furthermore, two separate procedures were followed to obtain the two solutions required for the determination of both major and trace elements. On the other hand both major and trace elements were analysed from the one solution produced by nitric acid oxidation (Garcia *et al.* 1972). This also reduced the amount of material required for analysis. This advantage was particularly important in the present work where the amount of material available was limited.

The determination of mineral elements, and particularly trace elements, in barley embryos, and in later studies, the endosperm, also requires extremely sensitive techniques since only very small amounts of material are available (1.0-12.0mg). Both flame and flameless AAS were found useful in analysing samples of this size.

Flame (air-acetylene) atomic absorption was used for the analysis of Mg, Ca and K. However, flameless atomic absorption, was used for the analysis of Mn, Fe and Cu. The levels of Mn, Fe and Cu in the embryo and endosperm may be as low as 0.005 μg and may not be detected by the more conventional flame methods of AAS. The technique of replacing the flame with a small electric furnace (carbon rod atomiser; CRA) developed by West & Williams (1969) and Massmann (1968) offers a highly sensitive analytical procedure (20x-500x more sensitive than the flame for most elements). In addition trace elements can be measured in plant samples as small as 1.0mg (Barnett & Kahn 1972) in microlitre volumes. Since a discrete volume of sample solution ($<5\mu$l) is vaporised as compared to the continuous spray (5cm^3) of flame analysis, wastage is virtually eliminated.

Metal ions may be lost by volatilization, or retained by forming insoluble salts, during the ashing and analysis procedures. To evaluate the method and assess such affects as reagent contamination, complete oxidation and inefficient atomization due to chemical interferences, the recovery of the elements under study was investigated.

3.2 Methods

The procedures described in sec. 2.7 and 2.9 were used for acid digestion and mineral ion analysis respectively, after the embryos and endosperms had been isolated from the intact grains. The recoveries of mineral elements were assessed as described in sec. 2.8.

3.3 Results

Instrumental Precision

In the initial assessment of instrumental precision, 4 concentrations each, of aqueous standards of all 6 metal ions were used. The within run coefficient of variation (cv) for Mg, Ca and K analysed with the flame were 0.5, 1.4 and 0.8 per cent respectively. Those of Mn, Cu, Fe with the CRA were 0.2, 0.1, 0.4 per cent. Number of determinations (i.e. n) was 10 in each case.

Recoveries

Table 3.1 shows the recoveries of individual metal ions added to embryo samples before digestion. The results of the same recovery experiment with endosperm samples are shown in table 3.2.

Table 3.1 The recoveries of K, Mg, Ca, Fe, Cu and Mn at three different added concentrations on acid digestion of 20, 30 or 40 days old embryos.

Element	Added conc. (mgl^{-1})	AGE IN DAYS 20 % Recovery	30 % Recovery	40 % Recovery
K	20.0	96.0 ± 4.2	105.0 ± 7.3	88.9 ± 6.0
	40.0	100.1 ± 5.0	108.3 ± 3.2	102.2 ± 2.1
	60.0	98.3 ± 1.0	96.4 ±10.0	95.6 ± 4.8
Mg	2.5	101.5 ± 7.3	107.7 ± 4.3	100.3 ± 5.3
	5.0	93.0 ± 2.3	99.4 ± 2.0	95.4 ± 1.0
	10.0	100.0 ± 1.0	98.3 ± 5.8	92.3 ± 8.3
Ca	1.0	101.0 ± 9.3	107.2 ± 2.4	106.7 ± 10.1
	3.0	94.5 ± 2.1	93.8 ± 5.6	88.8 ± 8.0
	5.0	87.3 ± 8.3	105.4 ± 1.3	90.3 ± 6.0
Fe	0.30	106.5 ± 4.0	105.5 ± 4.4	95.5 ± 11.1
	0.60	93.8 ±11.0	94.4 ± 0.0	107.7 ± 10.0
	1.20	95.6 ± 1.1	89.6 ± 5.2	101.3 ± 5.2
Cu	0.20	98.6 ± 6.0	106.6 ± 3.8	103.4 ± 8.8
	0.50	100.5 ± 7.2	94.6 ± 2.1	99.7 ± 2.1
	1.00	89.8 ± 3.3	99.7 ± 7.8	87.0 ± 4.9
Mn	0.10	100.0 ± 0.0	104.1 ± 2.3	103.1 ± 5.3
	0.20	98.4 ± 3.3	88.4 ± 2.1	98.9 ± 1.0
	0.30	96.8 ± 6.0	106.3 ± 1.0	95.0 ± 2.3

The recoveries represent means ± standard deviations from triplicate experiments.

Table 3.2 The recoveries of K, Mg, Ca and Mn at three different added concentrations on acid digestion of 20, 30 and 40 day old endosperm.

Element	Added conc. (mgl^{-1})	AGE IN DAYS		
		20 day old	30 day old	40 day old
		% Recovery	% Recovery	% Recovery
K	20.0	88.2 ± 0.0	90.1 ± 2.18	92.9 ± 10.1
	40.0	104.9 ± 3.5	91.7 ± 7.2	91.0 ± 5.2
	60.0	93.6 ± 6.0	97.9 ± 4.7	100.0 ± 1.8
Mg	2.5	85.3 ± 9.2	98.3 ±10.5	88.9 ± 11.0
	5.0	83.6 ± 0.0	92.3 ± 0.0	96.9 ± 2.2
	10.0	88.6 ± 4.0	94.3 ± 9.3	92.5 ± 3.2
Ca	1.0	89.7 ± 9.9	88.3 ±10.1	96.4 ± 5.5
	3.0	95.6 ± 1.0	84.8 ± 2.2	87.0 ± 4.7
	5.0	86.3 ± 3.2	88.1 ± 3.1	93.0 ± 7.3
Mn	0.10	96.3 ± 8.3	90.9 ± 0.0	94.7 ± 5.1
	0.20	100.7 ± 0.0	87.0 ± 0.0	89.9 ± 7.6
	0.30	91.2 ± 12.0	81.2 ± 2.4	83.3 ± 5.6

The recoveries represent means ± standard deviations from triplicate experiments.

3.4 Discussion

At the end of nitric acid ashing a clear solution was obtained which required no further filtration before analysis. Sources of contamination were kept to a minimum, since nitric acid, which is easily purified, was the only oxidising reagent.

Very good determinations of all the elements were obtained by AAS. Indeed this is probably one of the best techniques currently available for metal ion analysis particularly when they are present at very low concentrations. Methods of analysis for Mg as estimated by reproduceability were observed to be much better for atomic absorption than for the EDTA method (Pawluk 1967). Similarly, reproduceability using atomic absorption for Fe

determination was much higher than with the O-phenanthroline method. In the present investigation, matrix and chemical interferences were not significant with diluted ashed samples.

The within run cv for Ca was higher than the others at all the ages investigated. Chemical interferents like phosphate (PO_4), sulphate (SO_4), Fe and Al prevent complete atomization of Ca and lanthanum or strontium have been previously used to release Ca (Garcia *et al.* 1972, Pawluk 1967, Parker 1972). The ratio of P to Ca in the whole kernel is of the order of 10:1 and at these levels the interference effects are probably quite low. However, occasional difficulties were encountered in reproduceability of Ca and addition of La may reduce these problems. Duffus & Rosie (1976a) used La in the determination of Ca and Mg to eliminate interference from phosphate in the developing barley grain.

The method described was directly compatible with atomic absorption techniques which involve elemental standards in the concentration range of ug cm^{-3}. All six elements were analysed from just the one solution with a minimum of 5 embryos to a sample. Previous investigators used up to 75 barley embryos in order to detect trace elements (Duffus & Rosie 1976b) and Zook *et al.*(1970) used a wheat sample of 55g. Sample weights of 0.001-0.012g were routinely used in the present investigation.

Decomposing organic material without losing mineral elements is very important and few reports on the analysis of mineral elements indicate the extent of recovery of the elements on digestion. The good recoveries of all elements at the ages studied showed that both the ashing and analysis techniques used were sufficiently accurate for both embryos and endosperms. The techniques were also particularly suitable for the small sample size.

Since the chemical and physical composition of the embryo changes with development, it was necessry to evaluate the recovery of added elements at different ages. The results also showed that recoveries were essentially unchanged as the tissue grew and developed, in spite of associated alterations in the chemical and physical composition of the embryo. In addition chemical and matrix interferences did not change as development progressed. This could be due to the ratio of the interferent to the substance analysed remaining the same in spite of an increase in the absolute amounts of interferent present.

Although the good determinations observed with AAS are in broad agreement with previous work on mineral composition of cereals and cereal products (Garcia *et al.*1972 , Lorenz *et al.* 1974, Zook *et al*1970, Duffus & Rosie 1976 a and b); only Zook *et al.* (1970) have made an attempt to confirm the atomic absorption procedures developed by investigating the recoveries of elements studied. The recoveries of Mg, Mn and Cu amongst other elements in wheat and wheat products were reported to be 103±5.0, 102±2.0 and 99±7.0 per cent. However, hydrochloric acid was used to extract the mineral elements which is unsuitable for flameless AAS. Errors are introduced by the chloride forming a coating inside the carbon rod, and hence reducing the efficiency.

It is concluded that acid digestion with nitric acid provided a quick, simple and accurate preparative procedure for mineral element analysis using atomic absorption spectroscopy. The requirement for a very small sample size was particularly advantageous in investigations involving immature grain tissue.

4.0 Measurement and interpretation of barley embryogenesis.

4.1 Introduction

Before any study of biochemical changes during embryogenesis can be undertaken, a quantitative method must be devised for the measurement of embryo growth and development. The method should preferably be rapid and non-destructive and may include measurements of embryo fresh weight, dry weight or size. Measurement of changes in starch and protein content, being destructive, would not be suitable. Development may also be desribed in terms of chronological age (Merritt & Walker 1969) and morphogical changes occurring in the grain (Baxter 1972).

Fresh weight measurements can introduce errors due to water losses during excision and weighing of embryos. Embryo dry weight which increases throughout embryogenesis (Duffus & Rosie 1975, Cameron-Mills & Duffus 1979) would appear to be a good index. It is non-destructive (at least for metal ion analysis) and enables results obtained with greenhouse grown material to be compared directly with material grown under a variety of other conditions, including *in vitro* culture conditions. Size and length of the embryo may also be a satisfactory method of assessing developmental progress since it is rapid and simple. Using chronological age to define stage of development has its own limitations, in that age in days after anthesis is a function of the environmental conditions under which the plants are grown. Hence, morphological parameters may have to be included in an ageing system to avoid the considerable variation that may exist in developmental age between embryos from plants grown under different conditions.

In this study the changes in length, size and dry weight have been compared with morphological age in days after anthesis. The possibility of using a calibration curve to determine more complex parameters from simple measurements of size was also

investigated in the present work. For example, there may be a relationship between embryo length and dry weight such that any weights can be determined indirectly from simple measurements made non-destructively using a microscope.

4.2 Results

The growth curves of the barley embryos cv. Midas were sigmoid in shape when represented as increments in length, size and dry weight with time in days after anthesis (Figs. 4.1 and 4.2). The initial slow rate of growth in length and size up to 15 days after anthesis was followed by a faster exponential growth stage. Subsequently, the embryos more than doubled their length, after which the growth rate decreased remaining constant up to maturity. The maximum length attained at maturity was 3.2 ± 0.10mm under the conditions used for plant growth. During the exponential phase which continued up to 30 days, embryo size increased greatly, reaching a maximum of $8.9 \pm 1.1 mm^2$. During this period about 66 per cent of embryo length and 83 per cent of embryo size was achieved. Embryo width increased rather less markedly than length but made its biggest contribution during the exponential phase.

Similarly embryo dry weight, (Fig. 4.2) after an initial slow start increased exponentially between 20 - 32 days, the rate decreasing after about 32 days. The embryo reached a maximum dry weight of 1.9 ± 0.21mg at maturity. Fig. 4.3 shows the increase in embryo dry weight with size and the diauxic pattern of growth was again the predominant feature.

4.3 Discussion

The sigmoidal growth pattern observed with barley embryos was similar to that described for cotton (Reeves & Beasley 1935), beans (Walbot *et al.*1972), barley (Merry 1942, Duffus & Rosie 1975) and *Capsella* (Rijven 1952) embryos. It was notable that the

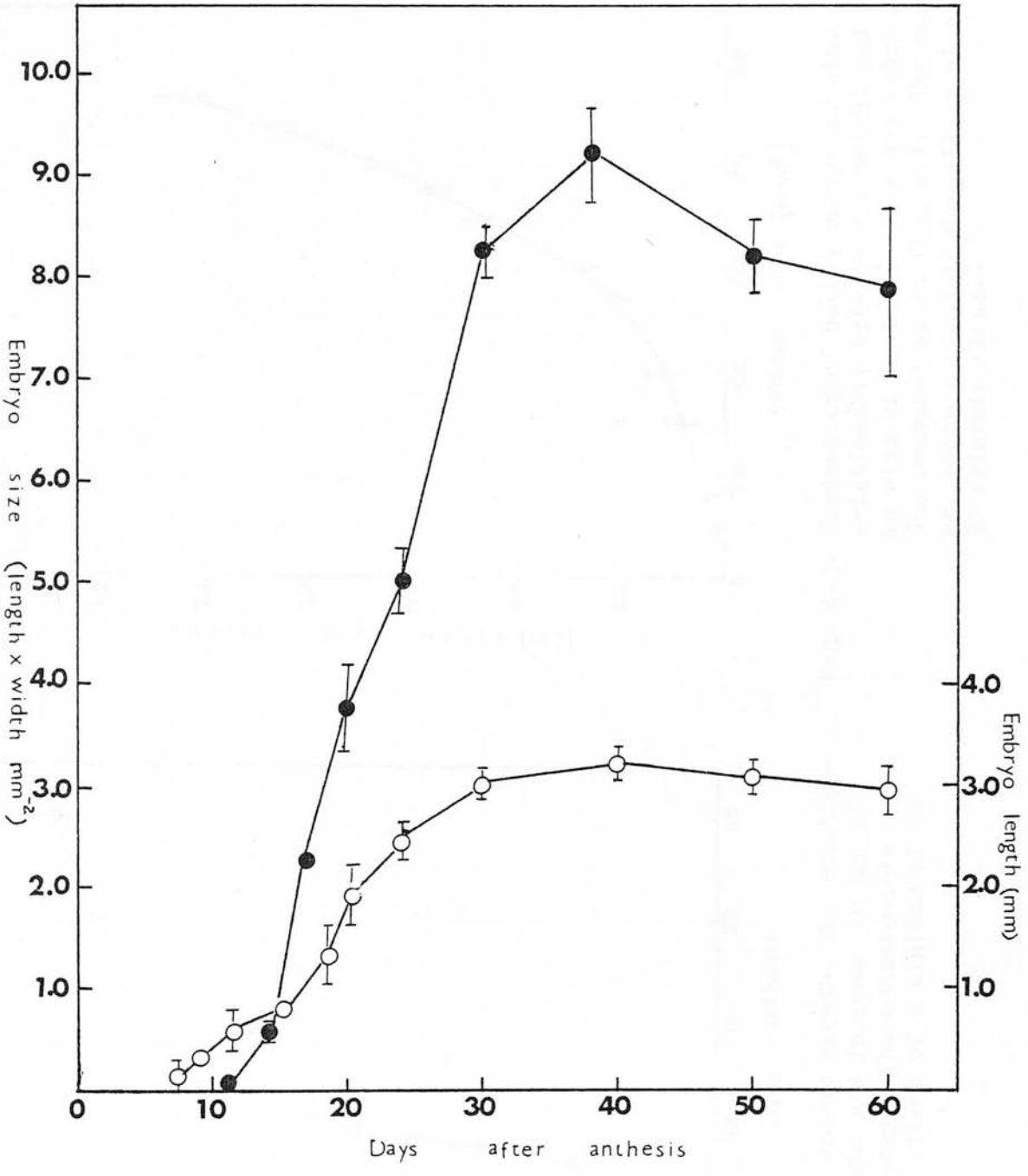


Fig. 4.1. Parameters of barley embryo growth. The relationship between length (○—○) and size (●—●) with increase in age of isolated cv. Midas embryos. Size was measured as a product of length and width, where length was measured between the scutellum perimeter and the coleorhiza tip. The measurements are means ± standard deviations of a minimum of 50 determinations. Plants were grown in greenhouse as described in sec 2. 2.

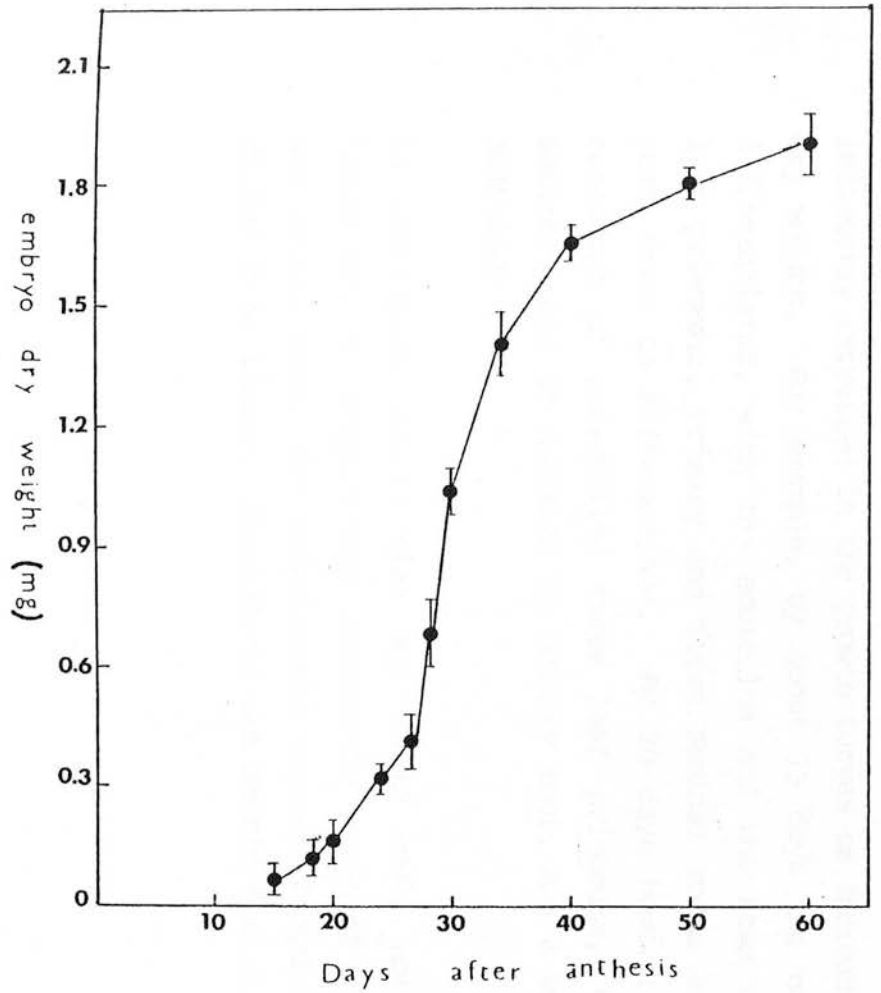


Fig.4.2. Parameters of barley embryo growth. The relationship between dry weight and increase in age of isolated cv. Midas embryos.The measurements are means ± standard deviations of a minimum of 50 determinations.

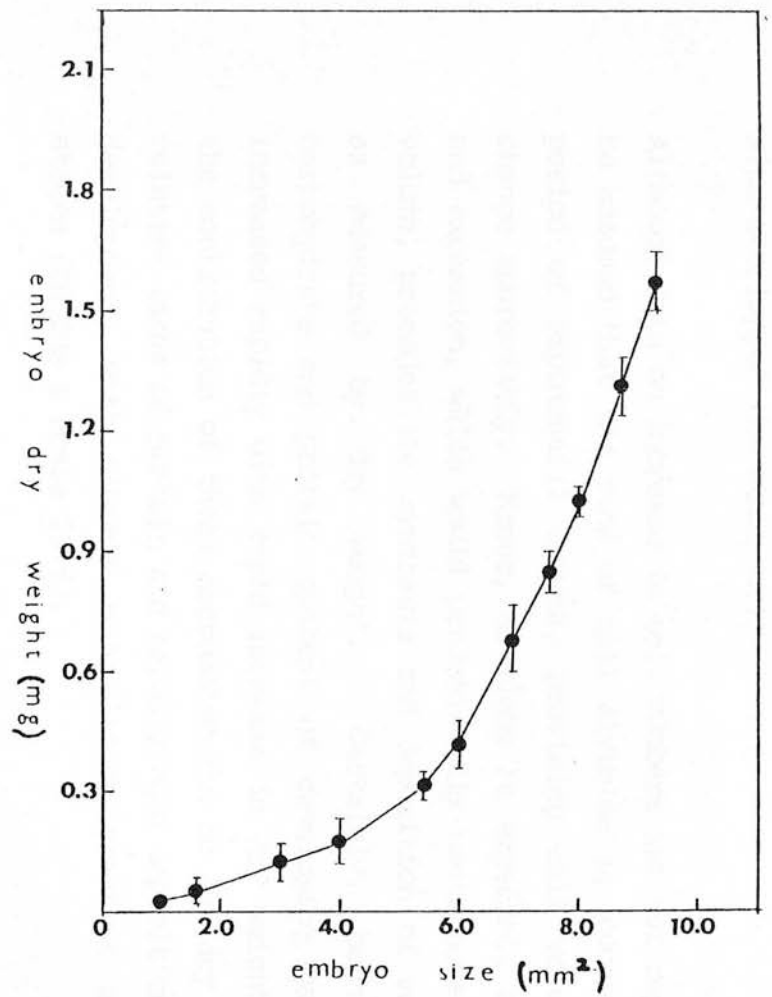


Fig. 4.3. Parameters of barley embryo growth. The relationship between dry weight and increase in size of isolated cv. Midas embryos. Size was measured as in Fig. 4.1. The measurements are means ± standard deviations of a minimum of 50 determinations.

rates of increase in length, weight and size were not synchronised. For example the greatest period of growth in length was observed about 10 days before the greatest period of growth in weight. Furthermore the period of greatest growth in size overlapped the other two.

Although data on increase in cell numbers are not reported it may be assumed that the rate of cell division is maximal during the period of exponential growth, providing cell volume does not change appreciably. Hence, as might be expected, cell division and expansion, which would predominantly contribute to size and volume, preceded the synthesis and deposition of macromolecules as measured by dry weight. Certainly, both the total carbohydrate and protein content of developing barley embryos increased rapidly with rapid increase in dry weight, confirming the contribution of these macromolecules to the dry weight. The relative rates of protein and carbohydrate deposition vary during development, with protein accumulation greatest at the later stages (Duffus & Rosie 1975).

Morphological changes during barley embryo development have been described (Merry 1941,) and the structural differentiation may be indirectly expressed in the growth curves as increase in size and dry weight. For example, by about 15 days the coleoptile was differentiated, with the scutellum and the stem meristem, two leaf primordia, primary and first seminal roots and coleorhiza just about to differentiate. By 30 days however, the embryo consisted of coleoptile, three leaf primordia, two pairs of seminal roots in addition to primary root, and a well developed scutellum.

In conclusion, embryo size and length could not be used as indicators of embryogenesis beyond the stage of dehydration of the grain, since the relationship between length/size and age ceased to be linear. Development was measured as size in *in vitro*

embryo culture since cultured embryos were not subjected to dehydration and only the linear part of the graph was used. Thus a simple non-destructive and rapid indicator of growth could be obtained by excision of embryos of one size from the whole grain. Both age in days after anthesis (either chronological or morphological) and dry weight were suitable indices throughout the developmental period, in particular for metal ion analysis. Morphological parameters (Baxter 1972) were used to establish the age of the grain when mineral elements were investigated during embryo development. However, chronological age (Merritt & Walker 1969) was required when Mg and Mn nutrition of the embryo was studied, since the varying concentration treatments may have influenced the morphological development of the grain.

5.0 The accumulation of mineral elements during barley embryogenesis

5.1 Introduction

Previous work (Duffus & Rosie 1976a, b) has described the variation in amounts of major and trace elements present in barley grain and its component parts during development (sec. 1.6). However, these authors did not indicate whether or not recovery of the extracted elements was complete, neither did they subject their data to tests of significance. Furthermore, trace element analysis was carried out using the less sensitive flame atomiser. Apart from this work, little is known of the role of mineral ions in developing embryos. Liu *et al.* (1975) have described the mineral content of developing and malted barley in five cultivars. However, analysis was limited to the whole grain over the developmental period. Mature grains were found to contain higher concentrations of mineral components in the embryo than in the starchy endosperm.

That mineral ions must be required for embryo development is clear from results demonstrating the presence of some enzymes in developing barley grain which depend on both major and trace elements for activity. Some of the enzymes involved in the Embden-Myerhof-Parnas pathway of glycolysis, requiring Mg for activity have been studied in the developing barley endosperm (Baxter & Duffus 1973, Duffus & Rosie 1977). These included the Mg-dependent phospofructokinase, hexokinase, pyruvate kinase and phosphoglycerate kinase. All the enzymes showed a pronounced peak of activity close to 35 days after anthesis and levels of activity fell to almost zero values by maturity.

Development in cereal grains is characterized by a net synthesis and deposition of storage materials such as starch and protein. Some of the enzymes e.g. nucleoside diphosphate kinase, of starch biosynthesis in the developing endosperm (Perez *et al* 1975) also

have an essential requirement for Mg. Starch synthase, another enzyme involved in starch biosynthesis was detected in barley endosperms (Williams & Duffus 1977). This enzyme which is activated by K has also been observed in maize embryos (Tandecarz *et al*1975).

Glutamine synthetase which has been detected in the endosperms and embryos of developing barley grains (Duffus & Rosie 1977) also has a specific requirement for Mg at physiological pH (O'Neal & Joy 1974). Fe is required for peroxidase activity (Dekock *et al*. 1960) and this enzyme has been detected in developing barley endosperms (Duffus 1970) and embryos (La Berge & Kruger 1976).

Mineral elements are also involved in the synthesis of DNA, RNA and protein. These macromolecules have been observed to accumulate in barley embryos during development (Duffus & Rosie 1975) and are presumably synthesised *in situ*. Mineral ions such as Mg, Ca and Mn are required to prevent dissociation of 70S ribonucleoprotein particles during protein synthesis in wheat and pea embryos (Lyttleton 1960). Additionally DNA and RNA polymerases are Mg - dependent enzymes (Mahler & Cordes 1971).

The work presented in this section describes the patterns of accumulation of three major elements - K, Mg and Ca and three trace elements - Fe, Cu and Mn during the development of barley embryos. The results are related to known structural and biochemical changes that occur during embryogenesis.

5.2 Methods

Embryos were dissected from the grains and samples prepared for mineral element analysis by wet ashing as described in sec. 2.7.1. K, Mg, Ca, Fe, Cu and Mn were analysed using conditions described in sec. 2.9. The changes in mineral ion levels in

developing barley embryos were expressed in terms of a range of different parameters:-

5.2.1 Amount of mineral element per embryo with increasing age after anthesis.

5.2.2 Amount of mineral element per embryo with increasing embryo dry weight.

5.2.3 Concentration of elements on a embryo dry weight basis (%w/w) with increasing age after anthesis.

5.2.4 Amount of mineral element per embryo as a percentage of the amount finally present in the mature embryo, with increasing age after anthesis.

5.3 Results

5.3.1 Fig. 5.1.1 shows the amounts of K, Mg and Ca per embryo at varying ages after anthesis. Both K and Mg had a sigmoidal pattern of accumulation with rates maximal between 20 and 30 days. The amounts of K and Mg were similar in the younger embryos (less than 18 days old) after which the curves diverged. The final amount of Mg present was 12 μg embryo $^{-1}$ and that of K was 22 μg embryo $^{-1}$. Ca levels were low and increased only very slowly over the whole period of development.

The patterns of trace element accumulation were, in general, very different from those shown by the major elements (Figs. 5.1.2). The exception was Mn where accumulation followed a sigmoidal pattern up to 25 days. Thereafter, accumulation was less rapid. The amounts of Fe and Cu increased rapidly after about 25 days, Fe accumulating at a greater rate than Cu. In contrast, the accumulation of these elements was linear with increase in age after anthesis.

Fig. 5.1.1. & 5.2.1. Variation in amounts embryo^{-1} of major elements K (■—■), Mg (●—●) and Ca (▲—▲).

5.1.2. & 5.2.2. Variation in amounts embryo^{-1} of trace elements Fe (○—○), Mn (□—□) and Cu (△—△).

The points represent means ± standard deviations from triplicate experiments. The standard deviations upto 25 days were < 5 per cent about the mean (Fig. 5.1.1. & 5.1.2.). The standard deviations upto embryo dry weight of 0.8 mg were < 8 per cent about the mean (Fig. 5.2.1. & 5.2.2.). Plants were grown under greenhouse conditions described in sec. 2.

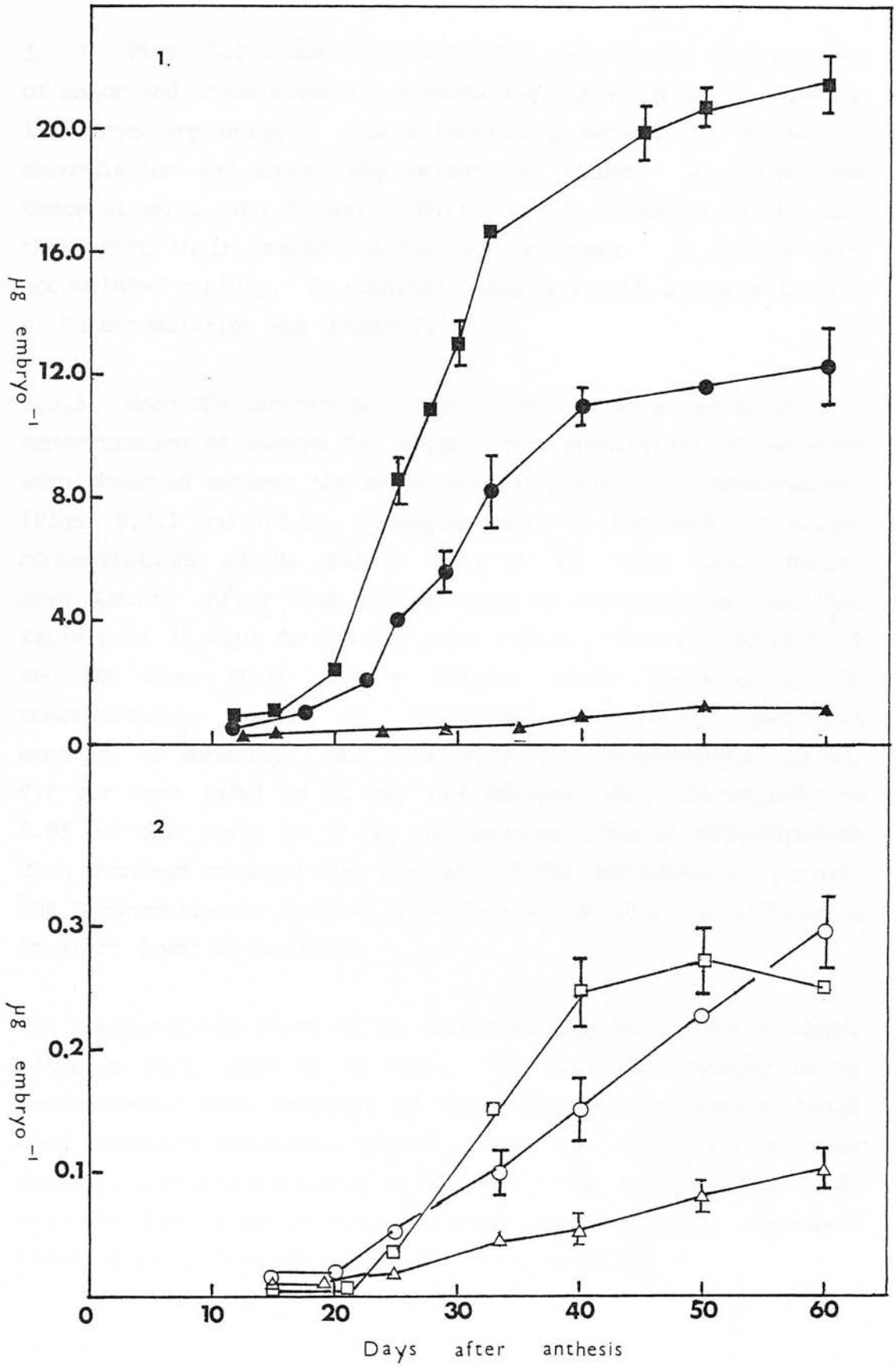


Fig. 5.1. The accumulation of mineral elemants during barley cv. Midas embryo development. The relationship between amount of mineral element embryo^{-1} with increasing age after anthesis. (see facing page for legend).

5.3.2 Figs. 5.2.1 and 5.2.2 show the patterns of accumulation of major and trace elements respectively, in relation to increase in embryo dry weight. The relationship between K, Mg and Ca accumulation and embryo dry weight was linear. As before the trace elements, Mn, Fe andCu initially all increased slowly, but thereafter their respective patterns diverged. Mn and Fe were accumulated rapidly. In contrast, only a slight increase in rate of Cu accumulation was observed.

5.3.3 When the amounts of mineral elements were expressed as a concentration of embryo dry weight then significant differences were observed between the early and later stages of development. (Figs. 5.3.1 and 5.3.2). Embryos about 12 days old had higher concentrations of Mg and Ca than at any other stage during development. After this initial peak Mg concentration declined rapidly at 15 days to 0.4 per cent (w/w). However, between 15 and 25 days there was a further small increase in Mg concentration, before it stabilised; thereafter remaining constant to maturity. The relatively high concentration of Ca, 0.6 per cent (w/w) in 12 day old embryos, declined rapidly to 0.05 per cent (w/w) in 20 day old embryos. The Ca concentration then remained constant for the rest of the developmental period. The K concentration reached a maximum at 20 days and achieved a constant level at maturity.

The concentration (w/w) of Mn increased rapidly beyond 22 days, after an early peak at 12 days. The relative increase in Fe concentration with increase in embryo dry weight between 15-30 days remained constant, beyond which the concentration rose rapidly, reaching a maximum at maturity. The concentration of Cu also declined from an early maximum but thereafter increased slowly from 25 days after anthesis until maturity.

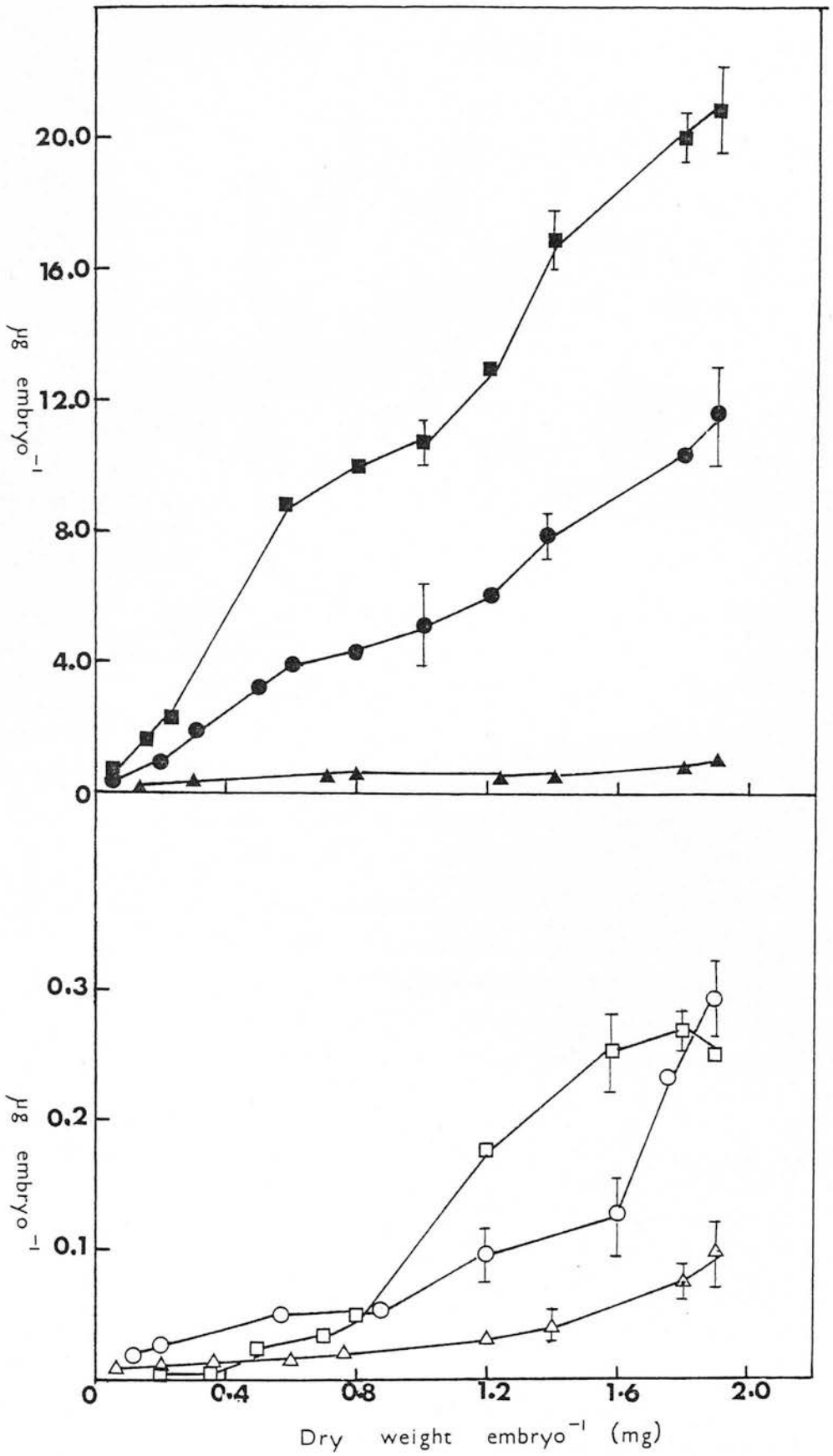


Fig. 5.2. The accumulation of mineral elements during barley cv. Midas embryo development. The relationship between amount of mineral element embryo^{-1} with increasing embryo dry weight. (See legend to fig. 5.1.).

Fig. 5.3.1. Variation in concentrations (w/w) of major elements, K (■—■), Mg (●—●), Ca (▲—▲).

5.3.2. Vaviation in concentrations (w/w) of trace elements, Fe (○—○), Mn (□—□), Cu (△—△).

The points represent means ± standard deviations from triplicate experiments. Plants were grown under green-house conditions as described for Fig. 5.1.

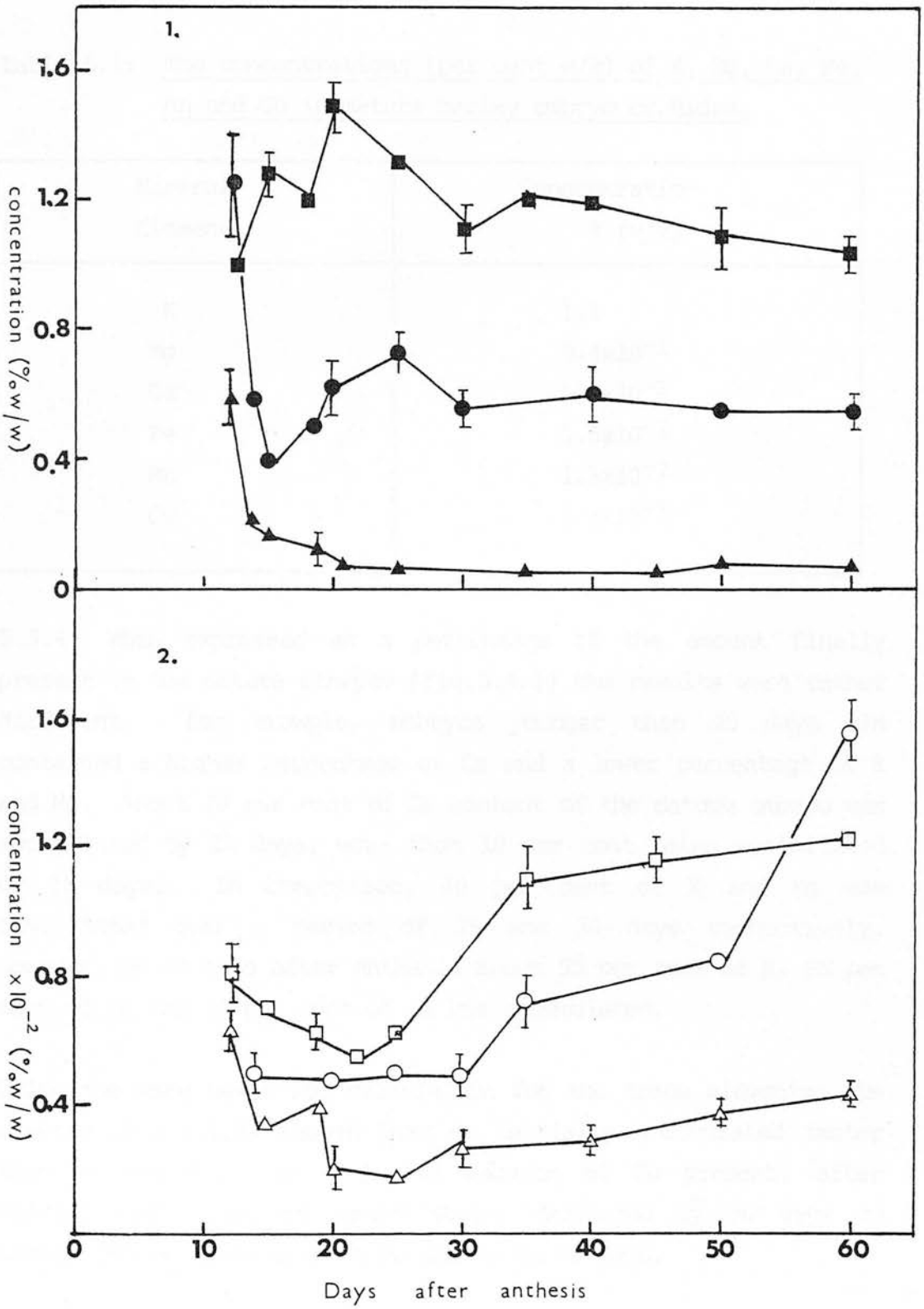


Fig. 5.3. The accumulation of mineral elements during barley cv. Midas embryo development. The relationship between concentration of elements on a embryo dry weight basis with increasing age after anthesis. (See facing page for legend).

Table 5.1 shows the concentration (per cent w/w) of K, Mg, Ca, Fe, Mn and Cu in the mature embryos.

Table 5.1: The concentrations (per cent w/w) of K, Mg, Ca, Fe, Mn and Cu in mature barley embryo cv. Midas.

Mineral Element	Concentration % (w/w)
K	1.1
Mg	5.4×10^{-1}
Ca	5.0×10^{-2}
Fe	1.6×10^{-2}
Mn	1.3×10^{-2}
Cu	4.5×10^{-3}

5.3.4 When expressed as a percentage of the amount finally present in the mature embryos (Fig.5.4.1) the results were rather different. For example, embryos younger than 25 days old contained a higher percentage of Ca and a lower percentage of K and Mg. About 40 per cent of Ca content of the mature embryo was accumulated by 21 days; more than 10 per cent being assimilated by 15 days. In comparison, 40 per cent of K and Mg was assimilated over a period of 25 and 30 days respectively. However, by 40 days after anthesis about 95 per cent of K, 85 per cent of Mg and 75 per cent of Ca was accumulated.

Using the same basis for calculation for the trace elements; the results (Fig.5.4.2) showed that Cu initially accumulated faster than Mn and Fe. The relative amounts of Cu present, after initial high rates of accumulation, decreased by 30 days to levels intermediate between Mn and Fe by 30 days.

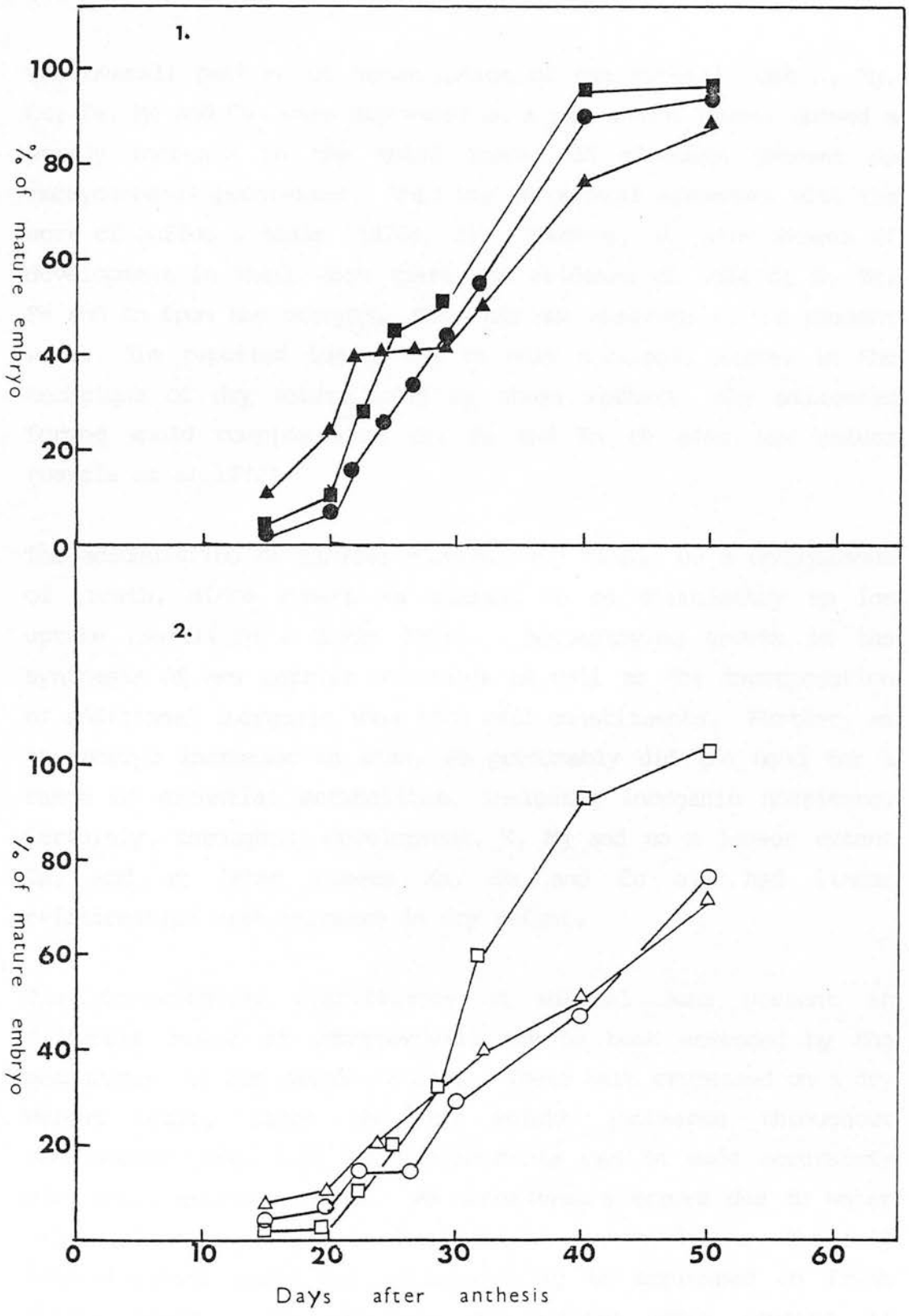


Fig. 5.4. The accumulation of mineral elements during barley cv. Midas embryo development. The amount of mineral element embryo^{-1} at a given stage as per cent of that in mature embryo (60 day old). 1. K(■—■), Mg(●—●), Ca(▲—▲). 2. Fe(○—○), Mn(□—□), Cu(△—△).

5.4 Discussion

The overall pattern of accumulation of the mineral ions K, Mg, Ca, Fe, Mn and Cu, when expressed on a per embryo basis, showed a steady increase in the total amount of elements present as embryogenesis progressed. This was in general agreement with the work of Duffus & Rosie (1976a, b). However, at later stages of development in their work there was evidence of loss of K, Mg, Fe and Zn from the embryos. This was not observed in the present work. The reported losses may be only apparent, since, in the technique of dry ashing used by these workers, the silicates formed would complex with Cu, Fe and Zn to give low values (Garcia *et al.*1972).

The accumulation of mineral elements may simply be a consequence of growth, since growth is claimed to be stimulatory to ion uptake (Sutcliffe & Baker 1976). Accompanying growth is the synthesis of new carrier molecules as well as the incorporation of additional inorganic ions into cell constituents. Further, as the embryo increased in size, so presumably did the need for a range of essential metabolites, including inorganic nutrients. Certainly, throughout development, K, Mg and to a lesser extent Ca, and at later stages Mn, Fe and Cu all had linear relationships with increase in dry weight.

The physiological significance of mineral ions present at different stages of embryogenesis may be best assessed by the measurement of ion concentrations. These were expressed on a dry weight basis, since the dry weight increases throughout development (sec. 4.3) and measurements can be made accurately with small embryo samples. As considerable errors due to water gain or loss may occur in fresh weight determinations, the cell concentrations could not satisfactorily be expressed on fresh weight basis. In addition, the embryo water content is influenced by age. It reaches a maximum at about 40 days (Duffus & Rosie 1975) and declines up to maturity.

Although there was an overall increase in mineral element content of embryos as they matured, high element concentrations (w/w) were observed in younger embryos; which then declined rapidly. However, if the moisture content of the embryo, which is higher at the earlier stages of development is considered, the concentrations (w/v) of the elements would then be lower as compared to concentrations on a dry weight basis at the earlier stages of development. The maximum concentration (w/v) would then be observed at maturity since the moisture content would then be very low. Lorenz & Reuter (1976) reported relatively high mineral concentrations (w/w) especially those of K and Ca, at the initial stages of grain development in several wheat, rye and triticale cultivars. Relatively high concentrations (w/w) of K in the early stages of barley embryo development were not observed in the present work. Since, the initial high concentrations declined very rapidly, it is possible that these relatively high concentrations of K were present in embryos younger than 12 days old. Similarly, a marked fall in the N and P concentrations (w/w) in the wheat grain during the first few days after anthesis has been observed (Dalling *et al.* 1976, Sofield *et al.* 1977).

The relatively high mineral concentrations at very early stages of grain and embryo development apparently occur as a result of a rapid uptake and redistribution by the plant. This may occur with any nutrient that is present in ample supply in readily available forms (Terman & Noggle 1973). At the later stages of grain development, dilution of the nutrient concentration and/or translocation may be the dominant trend, while nutrient uptake by the plant slows down. Liu (1975) observed large decreases in K and Ca concentration (w/w) in developing hulled barley and smaller decreases in P, Mg, Mn, Cu and Fe in naked barley grains during the second week after anthesis. This was attributed in part to the dilution effect, since the largest increase in grain weight also occurred at this stage.

As much as one per cent of K on a dry weight basis was present in the embryo over most of the developmental period. These relatively high amounts of K present in the embryo are difficult to correlate with the observed functions of K. Similar high requirements of plant cells for K have been described before (Evans & Sorger 1966, Bonner & Varner 1976).

The relationship between embryo growth and K accumulation may be similar to that described briefly for barley seedlings (Sutcliffe 1962; Pitman & Cram 1977 and Pitman 1975). The rate of K transport to these shoots was closely related to the relative growth rate and was independent of the transpiration rate or root/shoot ratio. Hence it appears that the content of K in plant tissues may be under metabolic control (Pitman 1975, 1977). During the period of greatest increase in dry weight (15 - 30 days after anthesis), the K concentration of the embryo increased and then declined to a constant level. This would be expected if K accumulation was related to growth.

Most of the dry weight increase is associated with the accumulation of carbohydrates, proteins and lipids. The total carbohydrate content of developing barley embryos increases steadily from 18 days onwards (Duffus and Rosie 1975) and indeed a major part of the young embryo is composed mainly of carbohydrates. K as mentioned above may have a role in enzyme - catalysed reactions during embryo development. The starch deposited in the developing embryo (Grewe & Le Clerc 1943, Duffus & Rosie 1975) which is a significant part of the total carbohydrate, is probably derived from precursor monosaccharides or disaccharides derived from the endosperm. The enzyme starch synthase, responsible for the synthesis of amylose and which may be present in developing embryos, has been reported to have a K requirement.

Protein accumulation in developing barley embryos was rapid beyond 25 days after a relatively slow start (Duffus & Rosie 1975). K was required for translation during protein synthesis and an optimum concentration range of 80 - 110 mM for *in vitro* translation in wheat embryos has been suggested (Wyn Jones *et al.* 1979). Rates of translation were much decreased at 200mM K.

Mg is known to play a major role in a number of biologically important mechanisms. The rates of increase of Mg accumulation in embryos was parallel to that of the other major cellular consituents after 30 days, so that the relative concentration remained constant to maturity. The increase in Mg concentration during the period of high metabolic activity (about 12-30 days) was probably due to its major role as a co-factor in numerous enzyme systems.

Ca may have a number of important functions during the development of the embryo. The relative concentration of Ca on a dry weight basis, in the young embryo was fairly high. Significant proportions of Ca in the embryo may occur as calcium pectate in the cell walls (El Hinnaway 1974). Or, it may be localized in the mitochondria, (Hanson & Koeppe 1975) and cell nucleus since it may have a role in cell division and in the organisation of chromatin associated with DNA structure (Hewitt & Smith 1975).

It is interesting to note that during periods of high Mg concentration when metabolic activity is high, as measured by protein/carbohydrate accumulation, Ca concentrations were low. This is of physiological importance since Ca has been reported to inhibit most enzymes stimulated by Mg (Bonner & Varner 1976, Mahler & Cordes 1971), possibly by competing at the binding sites. Ca also inhibits protein synthesis (Bonner & Varner 1976, Wyn Jones *et al.* 1979) and thus the low Ca concentrations may allow unimpeded action of Mg and K during embryo development.

That Mn can replace Mg in a range of biochemical processes particularly those of the glycolytic pathway, is well known (sec. 1.6.4). However, the concentration (w/w) of Mg was always considerably greater than the Mn concentration throughout embryo development (100 to 50 fold at maturity). Furthermore when Mg concentration was at its highest, Mn concentration was at its lowest. Hence, it may be that Mg was the main cofactor for these enzyme reactions during embryo development. It has been reported, however, that in addition to the Mg and Ca requirements for maintenance of ribosome integrity there is also a specific requirement for Mn in wheat and pea embryos (sec. 5.1). An additional function for Mn in developing embryos may be as a cofactor for RNA polymerase (Mazus & Brodinenicz-Proba 1976).

The rate of increase of Mn in the embryos at later stages of development is particularly high and this may be due to the increased requirement for Mn by the embryo or to the increased availability of Mn to the maturing embryo.

The accumulation of Cu in the developing embryo could be due to its requirement for respiratory metabolism. So far there is only indirect evidence for respiration in developing barley embryo, the presence of numerous mitochondria (plates 11.2 & 11.5). Since electron transport is localized in the mitochondria the absence or presence of mitochondrial cristae may indicate the extent of aerobic respiration. However, this may not be true for all tissues (sec.11.3).

More direct evidence for respiratory metabolism has been presented for developing cotton (Forman & Jensen 1965) and sunflower embryos (Prokof'ev & Rodinova 1966). The net oxygen uptake by cotton embryos increased with age, but when expressed on a per cell basis the oxygen uptake was highest in younger embryos. Oxygen uptake by extracted mitochondria of sunflower embryos decreased with age. In the present work it has been shown that excised immature barley embryos take up significant amounts of oxygen from a surrounding medium, thus indicating the presence of aerobic oxidation.

The increased uptake of Cu at later stages of development may be due to its requirement for plastocyanin synthesis. Certainly studies on ultrastructure of mature wheat (Setterfield *et al.* 1959, Swift & O'Brien 1972) and barley (Nieuwdorp 1963) embryos show the presence of plastids. These plastids may contain the photosynthetic units including plastocyanin, required by the seedlings after germination. However, in the above studies the shoot region of the embryo was not specifically examined and these plastids may not contain the photosynthetic apparatus.

The role of Fe in the embryo during development is probably mainly in the oxidation-reduction reactions of respiration (Bonner & Varner 1976). In addition to its structural role in the cytochrome oxidase complex (Chance *et al.* 1968), cytochrome c and b-cytochromes (Mahler & Cordes 1971), Fe may also have an affect on cell division in the embryo; probably involved in chromosomal structure (Hewitt & Smith 1975).

The accumulation of almost all the elements studied continued even at later stages in development. This was surprising, since the metabolic activity was minimal when measured by the accumulation of DNA, RNA and protein (Duffus & Rosie 1975). However, cereal embryos have significant mineral element reserves at maturity, located in the globoid crystals, and are possibly used during the first few hours of germination, before mineral ions such as K, Mg and Ca can be mobilised from the endosperm. For example, within the first hour following water imbibition both wheat (Spiegel *et al.* 1975) and barley embryos (Onckelen *et al.*1974) begin to synthesise RNA using Mg and Mn dependent RNA polymerase. Embryos of germinating barley also commence protein synthesis within the first 2h of germination (Stoddard *et al.* 1973). In addition, Fe and Cu reserves may also be required, since on imbibition, the respiration rate of dry embryos has been observed to rise rapidly.

Hence, although the relative concentrations of the elements examined appeared to be quite high at maturity, the concentrations of free ions in the cytoplasm were probably low, the elements being present as bound salts of phytic acid in the globoid crystals. This is of physiological advantage, particularly at maturity where the water content is low and elemental concentrations probably high. Such high concentrations could be toxic and result in tissue damage.

The comparison of the amount of mineral element at any stage to that present at maturity revealed the periods of the greatest and least accumulations of the elements. Ca was deposited earlier in embryo development than any of the other elements. This is not surprising since the decrease in transpiration during later stages of ear development reduces the amount of Ca reaching the maturing embryo. On the other hand most of Mn was deposited much later; Mn seems not to be required during early embryogenesis. The rest, more mobile elements, were accumulated rather more steadily over the developmental period.

In conclusion, although the accumulation of mineral elements was related to embryo growth, the concentrations present on a dry weight basis, were much higher at earlier stages of development than that at later stages. A large proportion of Ca present in the mature embryo was accumulated earlier on in development when the transpiration in the plant and ear was substantial. The greatest rates of accumulation of K, Mg, Cu and Fe occurred during the period of greatest embryo growth and development, the rate of accumulation slowing down at maturity.

6.0 The comparative accumulation of mineral elements during the embryogenesis of four barley cultivars.

6.1 Introduction

That the yield of a crop species varies according to cultivars is well established (Vose 1963). It is also well-known that a cultivar which gives the highest yield under one set of field conditions does not necessarily give the highest yield under all conditions. That the differential in yield between cultivars may be due to the efficiency in absorption of specific nutrients by plants was suggested by Lawrence (1944). Certain cultivars of barley were found to respond better to phosphate fertilizer than did others (Mitchell, 1957). Hayes (*loc.cit.* Vose 1963) found that Proctor barley responded significantly better to N fertilizer than Plum Archer.

Although there are many instances of differential nutrient uptake, (reviewed by Vose 1963) only in relatively few cases has the concentration of an element in the leaves or other plant part been related to differences in production between cultivars. Differences between cultivars in absorption of N, P, K, Ca and Mg by wheat grown under the same conditions have been demonstrated (Maume & Dulac 1943a, 1943b). Several other investigators (Kleese *et al.* 1968, Myers 1960, Sayre 1955) have reported differences in content of essential plant nutrients (P, K, Mg, Na, Ca, Mn, B and Sr) between cultivars of barley, wheat, soybean and corn. Significant differences were found in concentrations (w/w) of the elements studied in seeds and seedling leaves between cultivars of barley and wheat (Kleese *et al.* 1968). Rasmusson *et al.*(1971) also found large cultivar differences in concentration (w/w) of P, K, Ca and Mg in leaves and mature grains. Differences of up to 6.0 times in leaves and 2.5 times in grains were observed between 100 wheat and 150 barley

cultivars. However, in experiments comparing cultivar response to nutrient environment (P and K), differences among cultivars in grain and dry matter yield were not associated with cultivar differences in P or K content observed earlier in leaves or grains. Thus no evidence of genetic differences in the efficiency of utilization of P or K was obtained. However, these differences could have been masked by varying contents of other ions and their many interactions among them.

Some cultivars of a single crop species may have higher requirements for specific elements. Differences in susceptibility to Mn deficiency have been observed between cultivars of oats (Vose & Griffiths 1961, Vose 1963), and between cultivars of oats and wheat (Nyborg 1970). The mechanism of these differences may be very complex. For example Vose & Griffiths (1961) found in oats at maturity that the deficiency-susceptible cultivar Star had more Mn in the leaves than the resistant variety S171. The position as regards the roots was reversed, those of S171 having much higher Mn content than Star. On the other hand the differing susceptibility of pea cultivars to Mn deficiency was not accompanied by any corresponding differences in Mn content (Walsh & Cullinan 1945) and Nyborg (1970) suggested that greater sensitivity to Mn deficiency was caused by inability to take up Mn rather than to a higher requirement for the element.

Further, cultivars of a crop species may also show differential resistance to mineral toxicities. In a study on the effect of two levels of sodium chloride on different barley cultivars, Greenway (1962) found that at low levels grain yield was reduced only in the case of the susceptible cultivar. Cultivar differences in ion content reflected cultivar differences in growth and susceptible cultivars had higher chloride and Na and lower K than did resistant cultivars.

The mechanisms of cultivar differences which are not yet clearly defined can be summarized as variations in nutrient absorption, translocation and metabolism. The genetic basis of selective ion transport in plants has been discussed (Epstein & Jefferies 1964, Brown *et al*.1972). Differences in Mg absorption by the shoots of inbred lines of corn were attributed to differential immobilization of Mg in the stems, rather than to differences in the rate of absorption from the medium (Foy & Barber 1958).

Intentional selection for mineral content by the plant breeders is uncommon, at least in-so-far as improvement programs are concerned. This is probably because relatively little is known about the relationship between genetically determined differences in the accumulation of mineral elements and the capacity to produce economic yields.

Previous investigations involved in studying cultivar differences have determined mineral element content or concentration (w/w) of leaves, roots and dry seeds and in some cases differences in the concentrations have been related to cultivar differences. However, no attempt has been made before to determine any differences in patterns of mineral nutrition of the embryo. Indeed such studies could be used to evaluate cultivars variations without proceeding to the seedling stage.

In the present investigation the mineral nutrition (K, Mg, Ca, Fe, Cu and Mn) of four cultivars of Hordeum vulgare L.var distichum cv. Midas, Julia, Hassan and Zephyr grown under similar environmental conditions was studied. These are all spring barleys differing in their time of ripening. They ripen in the order Julia, Hassan, Zephyr and Midas. Both Julia and Hassan have a medium sized grain, whilst Zephyr has a larger and Midas a smaller grain. The malting qualities also vary from medium to good among the four cultivars. Mineral nutrition of the barley embryos was then studied to evaluate the differences, if any,

between the cultivars. Hassan has been shown to be more prone to Mn deficiency than the rest (Scottish Agricultural Colleges publication 1977). The accumulation patterns of Mn in addition to the other elements in the embryos of 4 cultivars were compared to see if any differences were evident in the uptake mechanisms for Hassan. In addition a survey of variation between a species would show whether or not results can be extrapolated from one cultivar to the other when grown under similar conditions.

Methods

All 4 cultivars were grown under greenhouse conditions as described in sec. 2.2. Ears were sampled periodically during development and stored at -18C until required. Embryos were excised and prepared for mineral element analysis as in sec. 2.7. Embryo length (sec. 2.4) and dry weights (sec.2.5) were also determined for each cultivar.

6.2 Results

The changes in dry weight of the four cultivars with increase in embryo length during development are shown in Fig 6.1. No significant differences were noted between dry weights of Julia, Hassan and Zephyr during development, but Midas embryos had significantly lower dry weight at several stages during development and at maturity.

The patterns of accumulation of K, Mg, Ca, Fe, Cu and Mn are similar in developing embryos of all four cultivars, (Figs. 6.2.1 - 6.2.6). The results are expressed as changes in the amount of mineral elements per embryo with increase in embryo dry weight.

The rate of accumulation of K was remarkably similar in all cases. The slopes of the graphs being 1.3, 1.3, 1.3 and 1.0 for Midas, Julia, Hassan and Zephyr respectively (Fig.6.2.1). Similarly those for Mg were 0.6, 0.6, 0.5 and 0.5 respectively (Fig.6.2.2). The amounts of K and Mg at maturity for the cultivars ranged between 22-25 μg embryo^{-1} and 9-12μg embryo^{-1} respectively.

Fig. 6.1. - Comparison of parameters of barley embryo growth of four barley cultivars. The relationship between the dry weight of isolated 1. Julia 2. Hassan 3. Zephyr and 4. Midas embryos and their increase in length. Length was measured as described for Fig. 4.2. The points represent means from triplicate experiments. All the standard deviations about the means were < 10 per cent. Plants were grown under green-house conditions described in Sec. 2.2.

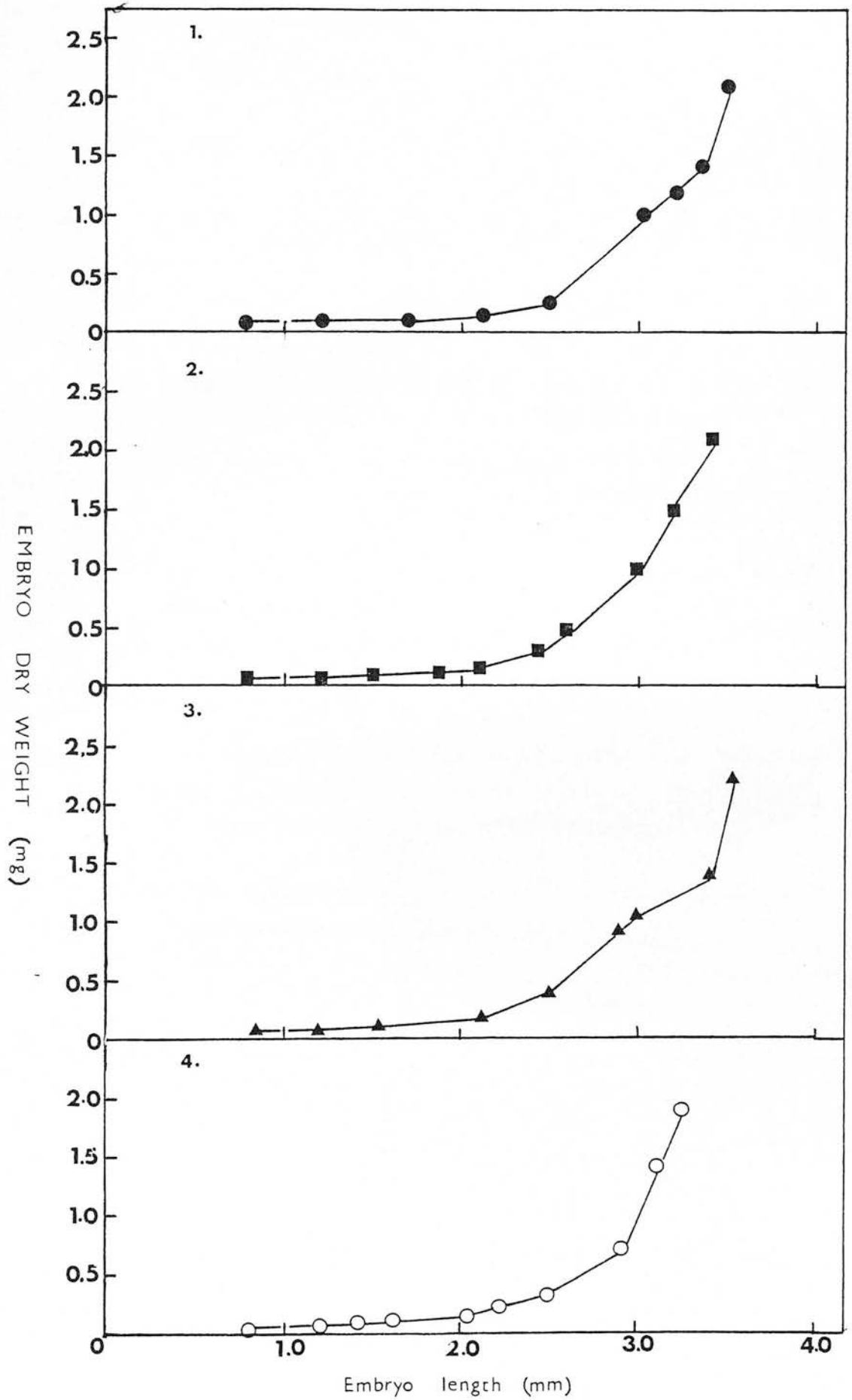

1.
2.
3.
4.
2.5
2.0
1.5
1.0
0.5
0
1.0
2.0
3.0
4.0
EMBRYO DRY WEIGHT (mg)
Embryo length (mm)

Fig. 6.2. Comparative accumulation of mineral elements during embryo development of four barley cultivars. Plants were grown under green-house conditions as described for Fig. 6.1.

Fig. 6.2.1. The variations in amounts of K . during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

Fig. 6.2.2. The variations in amounts of Mg during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

The points represent means from triplicate experiments. All the standard deviations about the means were < 12 per cent.

Fig. 6.2.1.

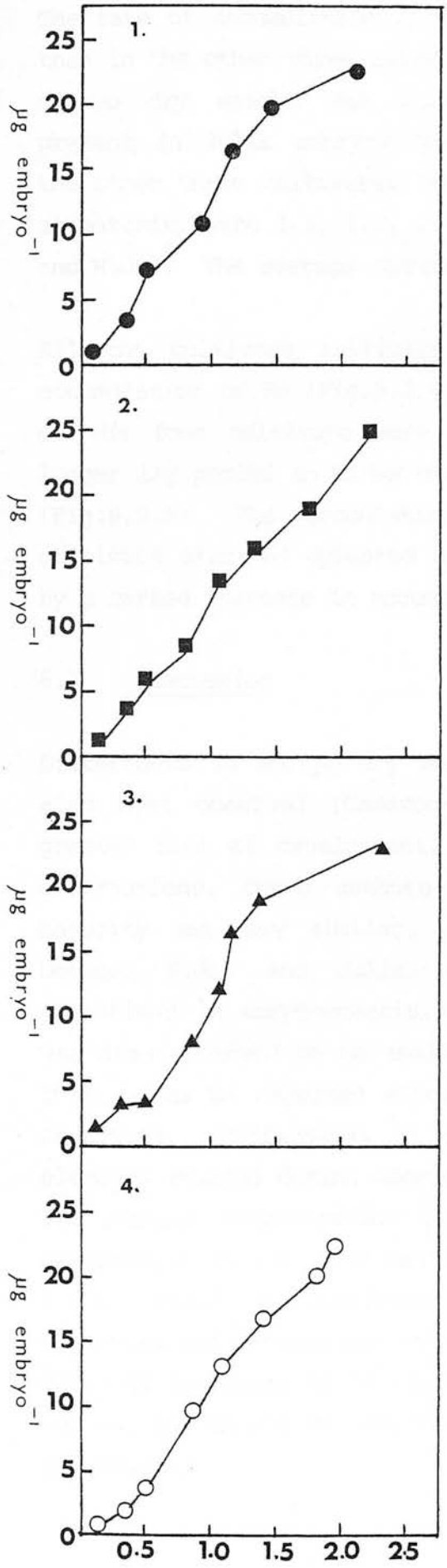
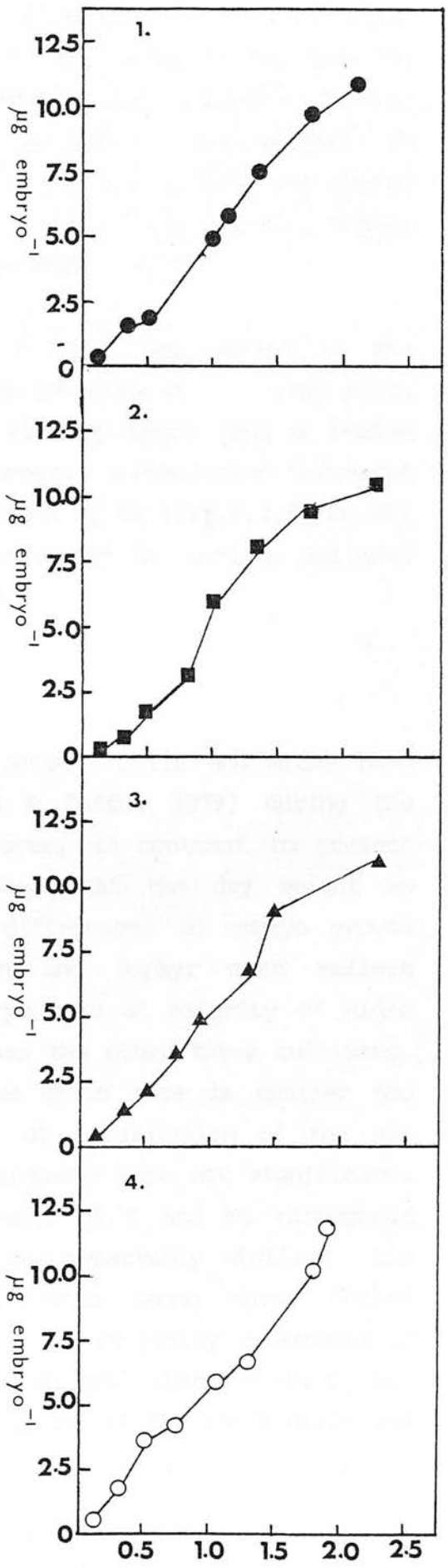


Fig. 6.2.2.



The rate of accumulation of Ca in Julia embryos was more rapid than in the other three cultivars (Fig 6.2.3) and by the time the embryo dry weight was 1.2mg, about 1.0µg embryo^{-1} Ca was present in Julia embryos compared to about 0.5µg embryo^{-1} in the other three cultivars. However, the levels of Ca per embryo at maturity were 1.5, 1.3, 1.4 and 1.2 for Julia, Hassan, Zephyr and Midas. The average standard deviation $\pm$ 0.25.

All the cultivars initially had a short lag period in the accumulation of Fe (Fig.6.2.4). The patterns of Cu accumulation of the four cultivars were also similar apart from a rather longer lag period in Midas before overall accumulation increased (Fig.6.2.5). The accumulation patterns of Mn (Fig.6.2.6) in all cultivars examined appeared as an extended lag period, followed by a marked increase in accumulation.

6.3 Discussion

Differences in embryo dry weight between Julia and Midas have also been observed (Cameron-Mills & Duffus 1979) during the greater part of development. However, in contrast to present observations, these authors reported that the dry weight at maturity was very similar. The differences in embryo growth between Midas and Julia, Hassan and Zephyr must reflect variations in morphogenesis. Embryo size at maturity of Midas was also observed to be smaller than the other three cultivars. This is to be expected since Midas grain size is smaller too (sec.6.1). Differences in trends of accumulation of the six elements studied during embryo development were not significant. The average concentration (w/w) range of K and Mg throughout development in all four cultivars was remarkably similar. Liu (1975) found no consistent differences among three hulled cultivars and between two naked cultivars of barley in amounts or rates of increases in the individual mineral elements (P, K, Mg, Ca, Mn, Cu, Zn and Fe) during development of the whole grain and at maturity.

Fig. 6.2.3. The variations in amounts of Ca during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

Fig. 6.2.4. The variations in amounts of Fe during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

The points represent means from triplicate experiments. All the standard deviations about the means were <20 per cent for Ca and ≤ 15 per cent for Fe.

Fig. 6.2.3.

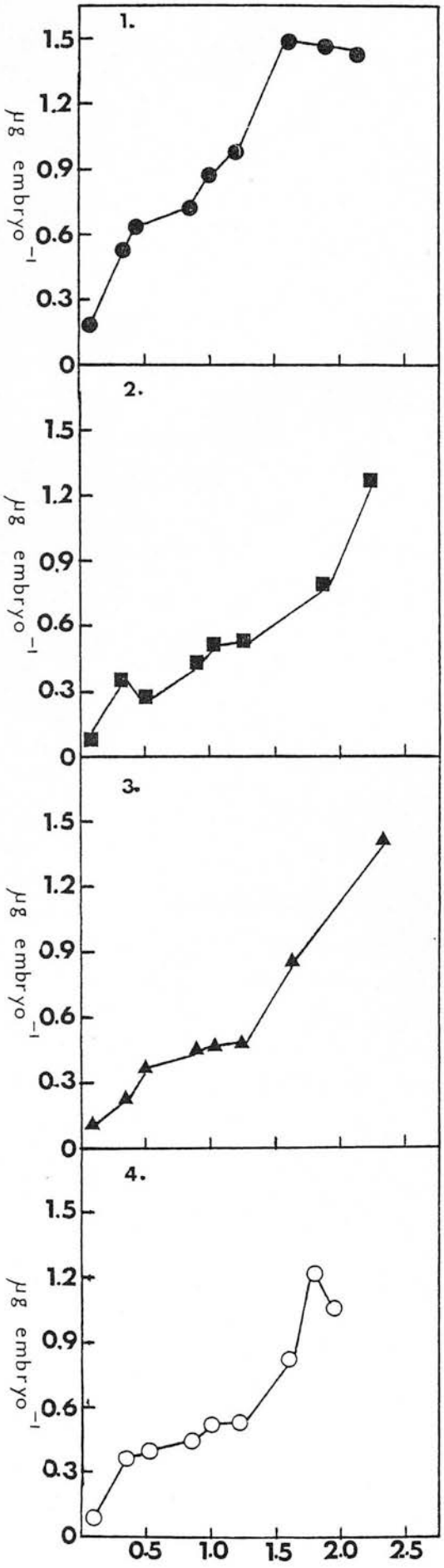


Fig. 6.2.4.

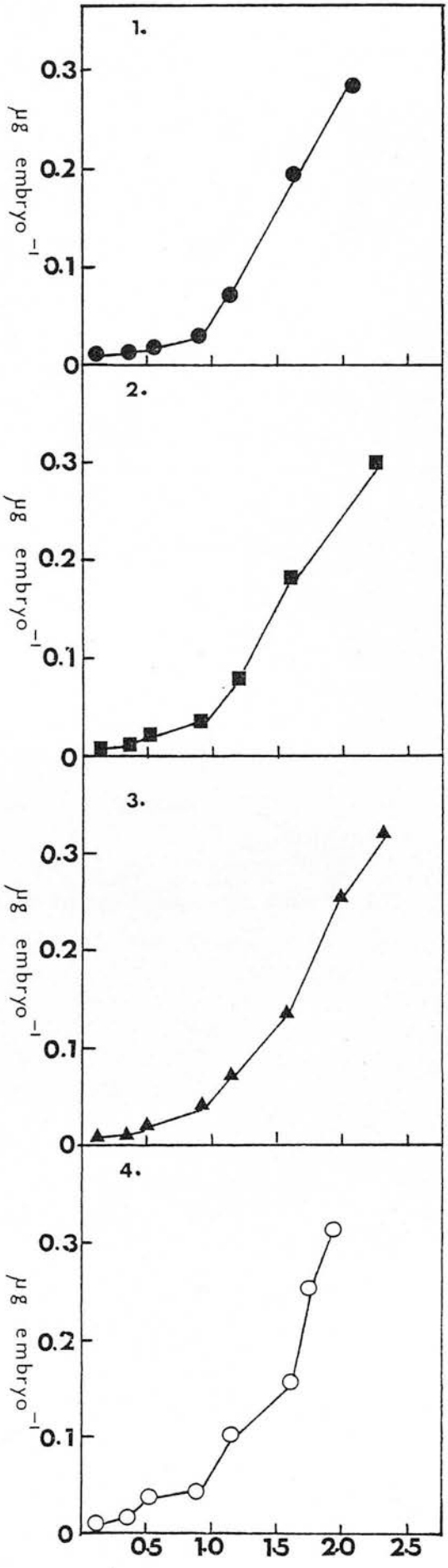


Fig. 6.2.5. The variations in amounts of Cu during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

Fig. 6.2.6. The variations in amounts of Mn during development of 1. Julia 2. Hassan 3. Zephyr 4. Midas embryos with increase in their dry weight.

The points represent means from triplicate experiments. All the standard deviations about the means were < 10 per cent.

Fig. 6.2.5.

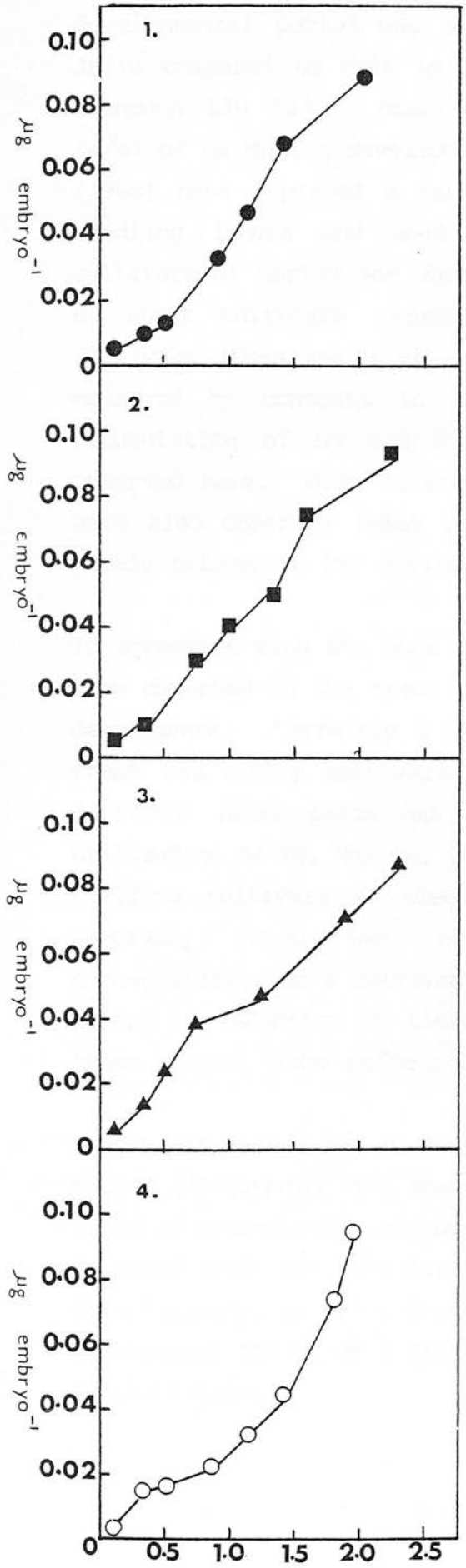
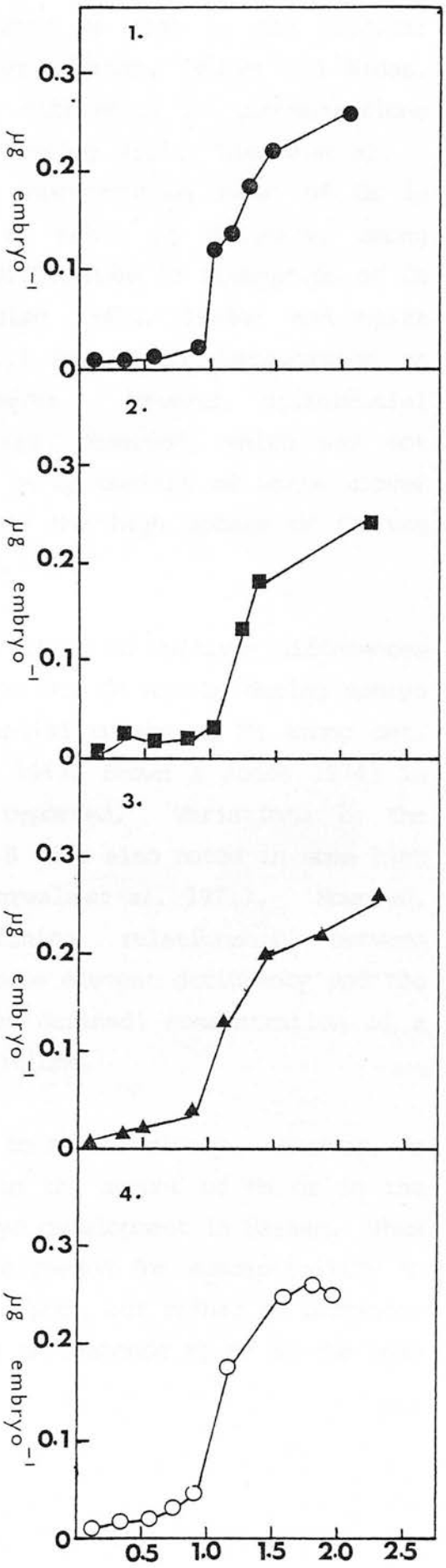


Fig. 6.2.6.



However, the concentration (w/w) of Ca during most of the developmental period was about twice as high in the cultivar Julia compared to that in cultivars Hassan, Zephyr and Midas. Although Liu (1975) observed no difference in concentrations (w/w) of Ca during development of barley grain; Kleese *et al.* (1968) have reported a range in concentration (w/w) of Ca in seedling leaves and seed samples taken at maturity, among cultivars of barley and wheat. Differences in absorption of Ca by wheat cultivars (Maume & Dulac 1943a, 1943b) and maize cultivars (Gorsline *et al.* 1961) have been demonstrated as measured by contents in the leaves. However, differential accumulation of Mg and K was also observed, which was not observed here. Wide differences in Ca content of white clover were also observed (Vose 1963) and the high uptake of Ca was partly related to low uptake of Mn.

In agreement with the work of Liu (1975) no cultivar differences were observed in the trace elements Mn, Fe and Cu during embryo development. Certainly a differential uptake of Mn among oat, wheat and barley cultivars (Vose 1963, Brown & Jones 1974) in different plant parts has been reported. Variations in the utilization of Fe, Mn, Cu, Zn and B were also noted in some high yielding cultivars of wheat (Agarwala *et al.* 1971). However, generally there was no definite relationship between susceptibility of a cultivar to trace element deficiency and the extent of reduction in tissue (not defined) concentration of a trace element under deficient conditions.

Hassan has been shown to be prone to Mn deficiency. However, no marked differences were observed in the amount of Mn or in the trend of accumulation, during embryo development in Hassan. Thus it would seem that, in Hassan, the reason for susceptibility to Mn deficiency, is not a decreased uptake, but rather an increased requirement for Mn or a difference in response to Mn at the same concentration.

Kleese *et al.*(1968) and Rasmusson *et al.* (1971) found differences between barley, wheat and soybean cultivars of approximately 1.2 to 1.4 fold for P, K, Mg, Mn and B; and 2.0 fold for Ca and Sr in seeds. No significant differences were found for Fe, Zn, Cu and Mo. The concentration (w/w) ranges in whole barley grains (12 cultivars) for K, Ca, Mg and Mn (Kleese *et al.* 1968) and for mature barley embryos for the four cultivars Julia, Hassan, Zephyr and Midas are compared below.

Table 6.1 Range in concentrations (w/w) of mineral elements in seeds and embryos of barley cultivars.

Element	Mature Seed (from Kleese *et al.*1968) (12 cultivars) % w/w	Mature embryos (4 cultivars) % w/w
K	0.513 - 0.683	0.98 - 1.27
Mg	0.110 - 0.141	0.41 - 0.53
Ca	0.040 - 0.071	0.05 - 0.08
Mn	0.015 - 0.019	0.010 - 0.013

The concentration ranges of the elements between cultivars for seeds and embryos were similar. Kleese *et al.* (1968) concluded these ranges to represent genetic differences (significant at 1%) in mineral element contents of seeds and hence the ranges observed in the embryos may also imply a difference between the 4 cultivars. However, in the absence of sufficient replicates both under field and greenhouse conditions, the significance of the differences in concentration between the embryos can not be established.

In conclusion, the patterns of mineral ion accumulation during embryo development in the cultivars studied were similar, although the concentration (w/w) of Ca was rather higher in Julia. In addition, since the Midas embryo weighs less, the absolute concentrations of Mn may be higher. To establish whether or not genotypic variations exist it is necessary to study higher numbers of cultivars under a wide range of environments.

7.0 Effect of variation in the Mg supply on embryo and endosperm development during grain maturation.

7.1 Introduction

The effects of varying mineral element concentrations including Mg, on grain development have received little attention, although these effects are well documented for most essential elements during the early or vegetative growth of many plants. This may be due to difficulties in obtaining plants which set seed and grow under stress conditions to maturity. Mg is present in relatively high amounts throughout the development of barley embryos (sec.5.3) and endosperm (Duffus & Rosie 1977), hence its importance during grain development can not be ignored.

In order to study the effects of depletion of Mg on barley grain tissues, barley plants were grown under varying Mg concentrations. Since it is impossible to manipulate the mineral nutrient supply to plants grown in soil, plants were grown in a soil-less, solid rooting medium, in this case, sand. The inert properties of sand make it possible to use a defined mineral nutrient solution which can be easily varied for differing Mg treatments. Sand, and not water culture, was selected as the basis of this experiment since it provides a natural support for the roots of the plant and is hence closer to natural soil conditions. Furthermore sand culture plants suffer less from chlorosis than those grown in water culture where pH changes may reduce the availability of some minerals e.g. Fe. In addition simple gravitational drainage allows for good aeration; lack of which may affect nutrient absorption and pH around the roots. On the other hand, changes in nutrient treatment during the period of an experiment can be more easily made by replacing solutions in water cultures, leaching the sand with distilled water may not always be effective.

Selected essential mineral elements were analysed in the embryos and endosperms during grain development from the plants grown in sand culture. The effects of Mg depletion in the rooting medium were also studied into the next generation. Since the role of Mg in chlorophyll is well documented, the deficiency symptoms of chlorosis provided an effective marker of the Mg status of the seedlings.

7.2 Methods

Plants were grown in sand culture (sec. 2.10.2) and Mg withdrawn at anthesis as described in sec. 2.10.2.1. Ears at different stages of development were harvested 30, 40 and 50 days after Mg withdrawal. K, Mg, Ca and Mn contents were determined in embryo and endosperms (sec.2.7) of each treatment. The concentrations were expressed as a per cent of dry weight. The effects of varying Mg supplies on these concentrations were studied with increase in embryo age. The results were compared with control plants which were supplied with Mg throughout the growth period. The stage of development of ears at the time of Mg withdrawal was also recorded.

Fresh and dry weight measurements of grains, endosperms and embryos during development were obtained for harvests at 30, 40 and 50 days and compared between treatments. Seeds harvested at maturity from -Mg and control plants were sown in washed sand with no further mineral nutrient supply, and the seedlings examined. Chlorophyll determinations in leaves were carried out as described in sec. 2.10.5. Ultrastructural studies on these leaves were performed using transmission electron microscopy (sec. 2.12.2).

7.3 Results

7.3.1 General observations

Leaves from plants 3 - 4 weeks after germination showed signs of blue-green colorations. However, the mature plants displayed no peculiarities. On average, the straw length was smaller than those grown in soil under similar greenhouse conditions. Older plants had slightly high numbers of brown specks on leaves than plants grown in soil. Tillering in all three treatments was prevalent and frequent periodic counts (not shown) showed no significant differences in numbers of tillers formed. The number of grains filled per ear was not significantly different between the treatments.

Fig. 7.1 shows the fresh and dry weights of all embryos sampled during the complete course of the experiment i.e. harvesting at 30, 40 and 50 days for the control levels of Mg (1.5mM). The embryo weights at different ages on any ear harvested 30 days after the start of treatments were higher than the corresponding weights of embryos of the same age on ears harvested after 40 and 50 days. Similar patterns of weight were observed in the sampling of -Mg embryos during the complete course of the experiment (Fig. 7.2).

The results presented in the following sections will exclude those obtained from the intermediate Mg treatment 0.3; (1/5 of control concentration), in order to obtain clear graphical presentation. In all cases the weights and mineral element concentrations recorded lie between the two extreme treatments i.e. no Mg and the control Mg level.

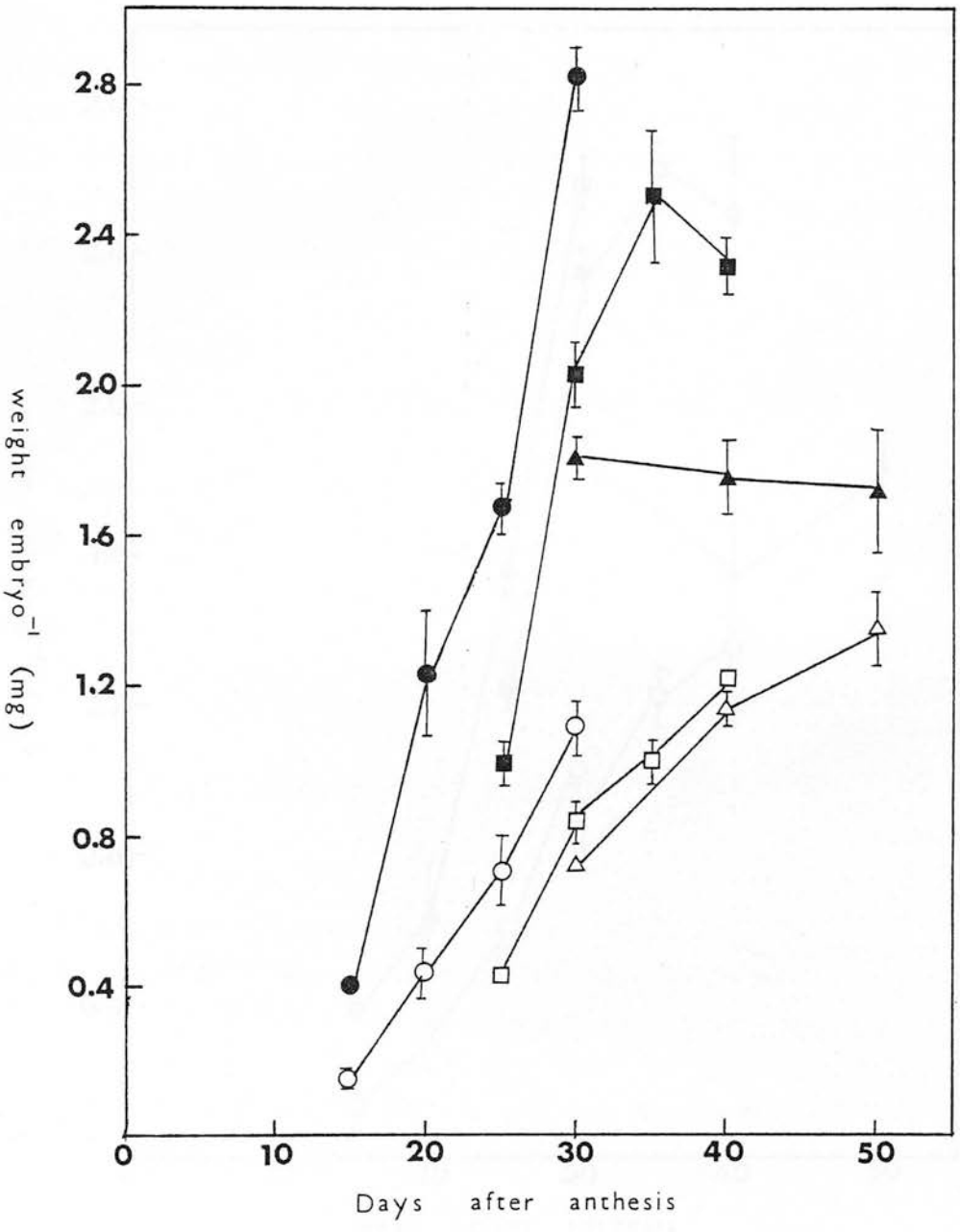


Fig. 7.1 Variation in embryo weights during sampling of plants grown in sand culture. Fresh weights (closed symbols) and dry weights(open symbols) of developing embryos; harvested from control plants at 30 (●—●) 40(■—■) and 50(▲—▲) days after withdrawal of Mg. The points represent means ± average errors from two experiments. Plants were grown as described in sec. 2.10.

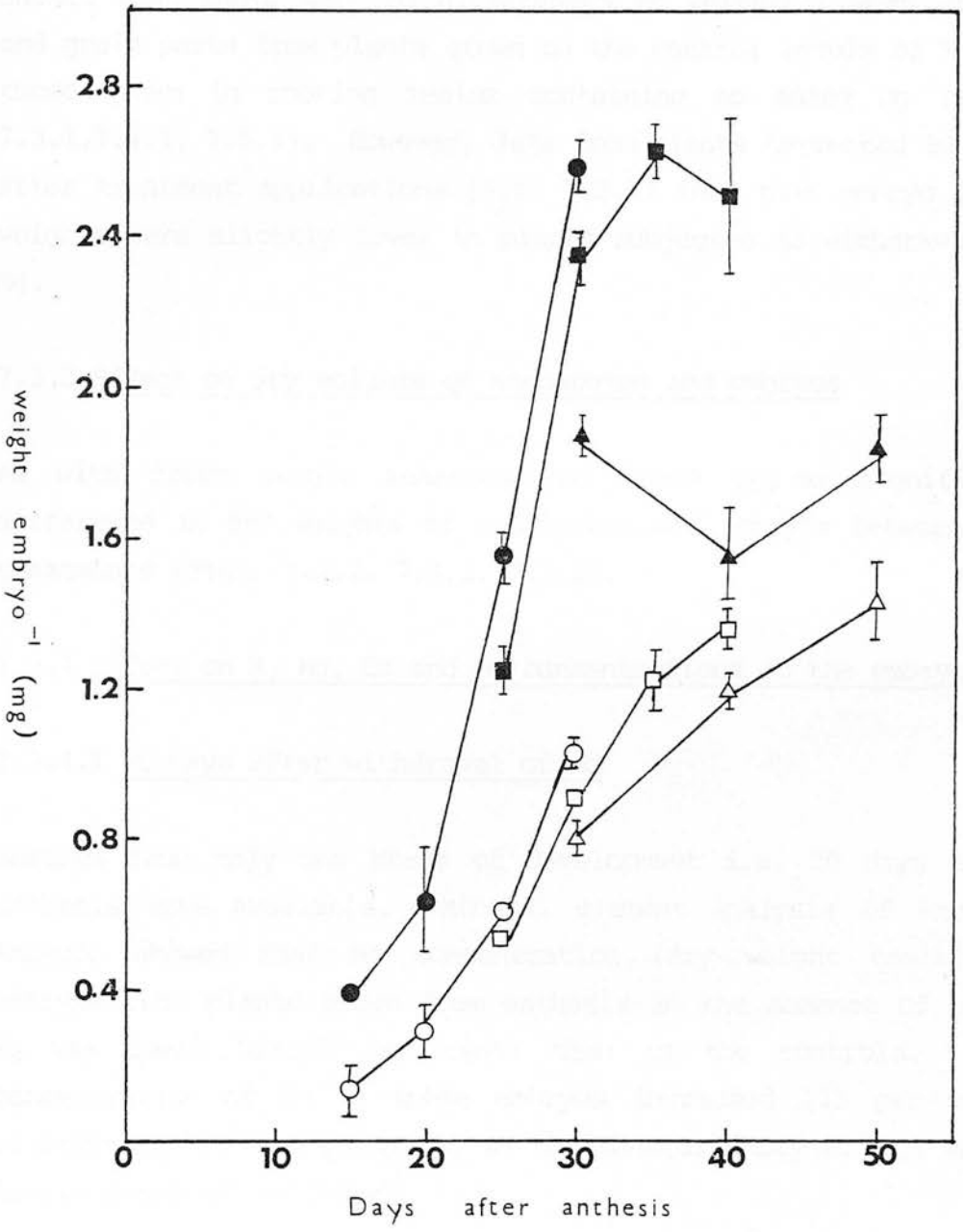


Fig. 7.2. Variation in embryo weights during sampling of plants grown in sand culture. Fresh weights (closed symbols) and dry weights (open symbols) of developing embryos: harvested from Mg depleted plants(-Mg), at 30(●—●) 40(■—■) and 50(▲—▲) days after withdrawal of Mg.

The points represent means ± average errors from two experiments. Plants were grown as described for Fig. 7.1.

7.3.2 Effect on fresh weights of intact grains, endosperms and embryos

Fresh weight measurements of intact grains, endosperms and embryos harvested at 30, 40 and 50 days after Mg withdrawal showed that there were no differences in weights between grains and grain parts from plants grown on the control levels of Mg and those grown in rooting medium containing no added Mg (Figs. 7.3.1,7.4.1, 7.5.1). However, data from plants harvested 30 days after treatment applications (Fig. 7.3.1) show that embryo fresh weights were slightly lower in plants subjected to withdrawal of Mg.

7.3.3 Effect on dry weights of endosperms and embryos

As with fresh weight measurements, there was no significant difference in dry weights of endosperms and embryos between the treatments (Figs. 7.3.2, 7.4.2, 7.5.2).

7.3.4 Effect on K, Mg, Ca and Mn concentrations in the embryo

7.3.4.1 20 days after withdrawal of Mg

Samples from only one stage of development i.e. 20 days after anthesis were available. Mineral element analysis of excised embryos showed that Mg concentration (dry weight basis) in embryos from plants grown from anthesis in the absence of added Mg was lower (ca. 17 per cent) than in the controls. The concentration of Mn in these embryos increased (33 per cent) relative to the concentration in the control embryos. Ca and K levels remained unchanged.

7.3.4.2 30 days after withdrawal of Mg

The concentration of Mg in embryos from -Mg plants was also lower than the control levels, at every age examined (Fig. 7.6.1). The depleted embryos from ears between 15 - 25 days after

Figs.7.3.1, 7.4.1., & 7.5.1.

Fresh weights of grains (●—●), endosperms (■—■) and embryos (▲—▲) from control (closed symbols) and Mg withdrawn plants (open symbols).

Figs.7.3.2., 7.4.2., & 7.5.2.

Dry weights of endosperms (■—■) and embryos (▲—▲) from control (closed symbols) and Mg withdrawn plants (open symbols).

The points represent means ± average errors from two experiments.

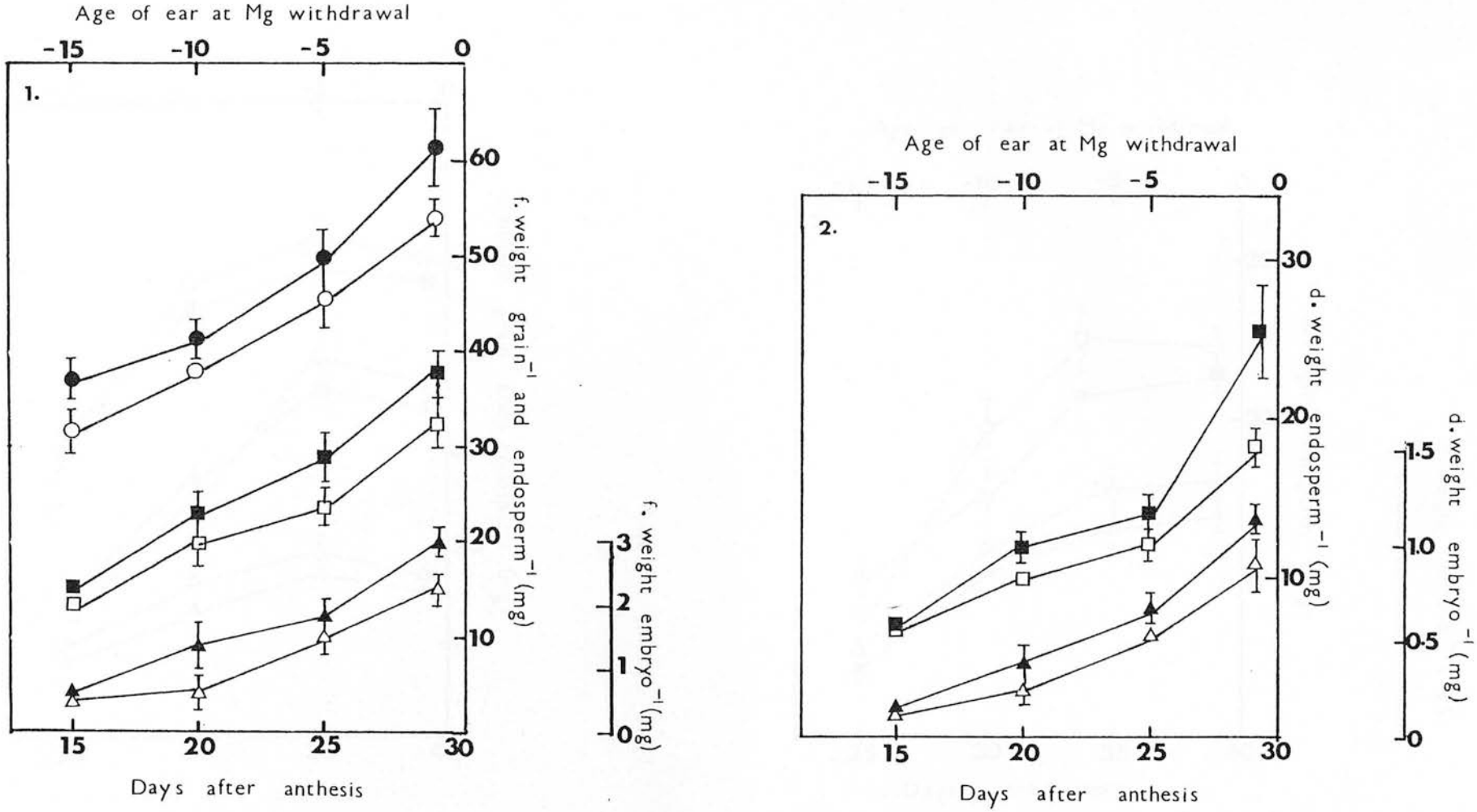


Fig. 7.3. Effect of variation in the Mg supply on tissue weights during grain development. Samples were harvested 30 days after Mg withdrawal from plants grown in sand culture as described in Sec. 2.10. (See facing page for legend).

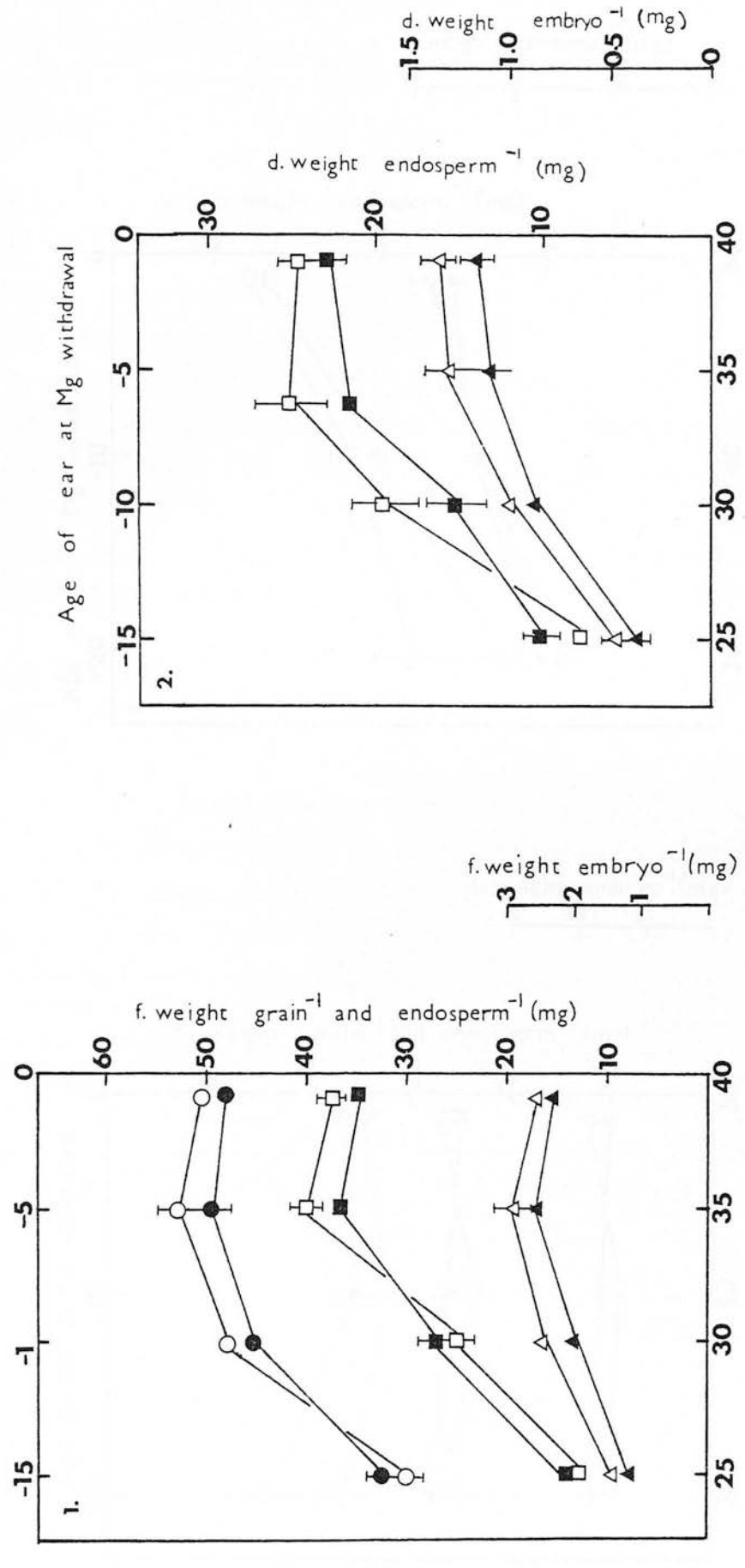


Fig. 7.4. Effect of variation in the Mg supply on tissue weights during grain development. Samples were harvested 40 days after Mg withdrawal, from plants grown in sand culture as described in Sec. 2.10. (See legend to Fig. 7.3.).

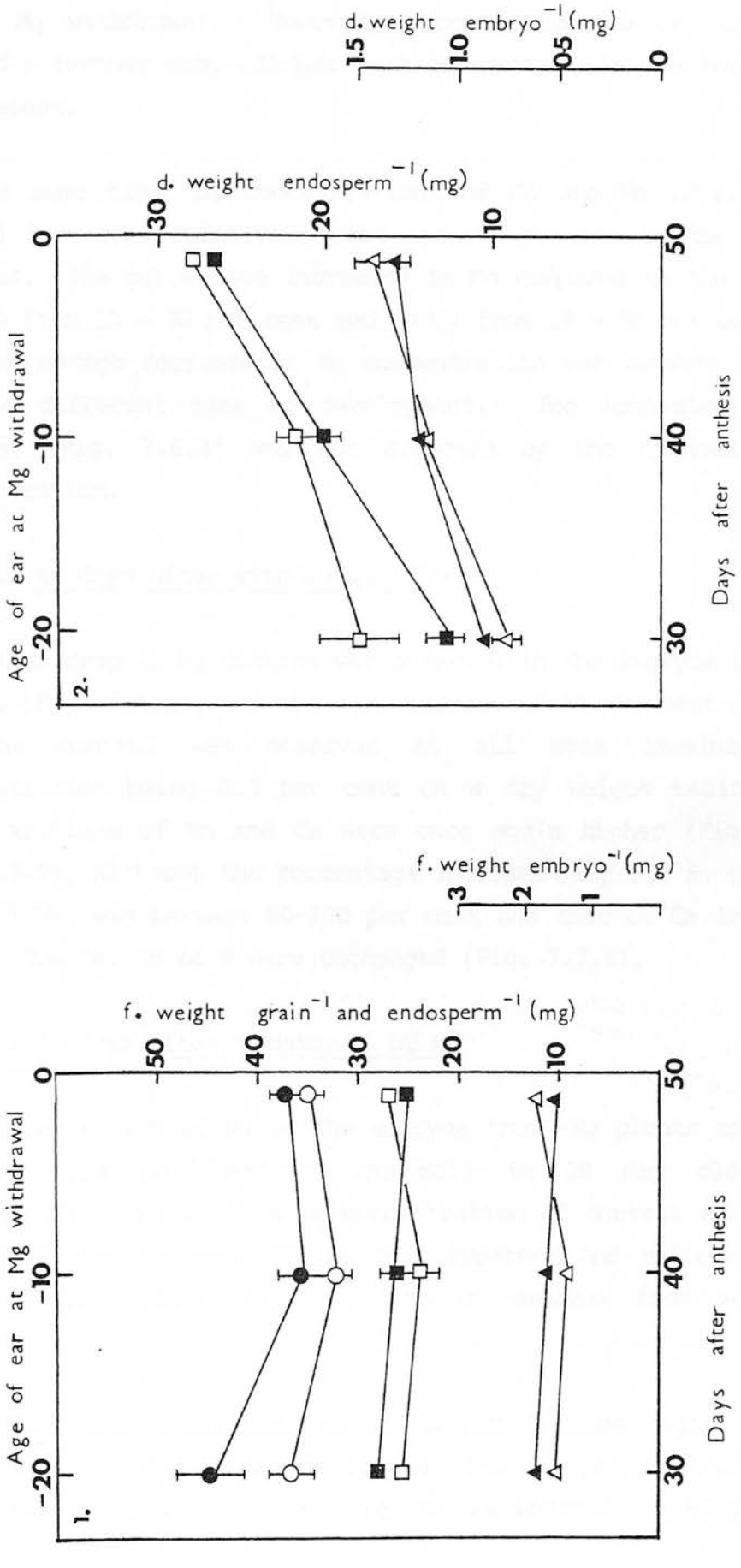


Fig. 7.5. Effect of variation in the Mg supply on tissue weights during grain development. Samples were harvested 50 days after Mg withdrawal from plants grown in sand culture as described in sec. 2.10. (See legend to Fig. 7.3.)

anthesis contained levels similar to those in embryos 20 days after Mg withdrawal. However, embryos from 30 day old ears showed a further drop (33 per cent of control) in the amounts of Mg present.

At the same time the concentrations of Ca and Mn (Fig. 7.6.2, 7.6.3) increased relative to the amounts present in the control embryos. The percentage increases in Mn compared to the control ranged from 33 - 50 per cent and in Ca from 25 - 50 per cent when the percentage decrease in Mg concentration was between 16 - 33 at the different ages of development. The monovalent K ion content (Fig. 7.6.4) was not affected by the changes in Mg concentration.

7.3.4.3 40 days after withdrawal of Mg

A further drop in Mg content was observed in the embryos from -Mg plants (Fig. 7.7.1). An average decrease of 50 per cent compared to the control was observed at all ages examined, the concentration being 0.3 per cent on a dry weight basis. The concentrations of Mn and Ca were once again higher (Fig. 7.7.3 and 7.7.2), although the percentage increase compared to the control in Mn was between 80-100 per cent and that of Ca 18-28 per cent. The levels of K were unchanged (Fig. 7.7.4).

7.3.4.4 50 days after withdrawal of Mg

The concentration of Mg in the embryos from -Mg plants continued to drop (59 per cent of control) in 30 day old ears, (Fig.7.8.1). The fall in Mg concentration of control embryos in 40 and 50 day old ears may be only apparent and reflect errors arising from incomplete dissection of embryos from very dry grains.

As before, the concentrations of Mn and Ca were higher in the embryos from Mg depleted plants (Fig. 7.8.3, 7.8.2) the percentage increase in Mn compared to the control was 93 per cent

Figs. 7.6., 7.7., & 7.8.

Variations in concentrations of 1. Mg (●—●) 2. Ca (▲—▲), 3. Mn (■—■) and 4. K (▼—▼) in developing embryos from control plants (closed symbols) and from Mg withdrawn plants (open symbols).

The points represent means ± average errors from two experiments.

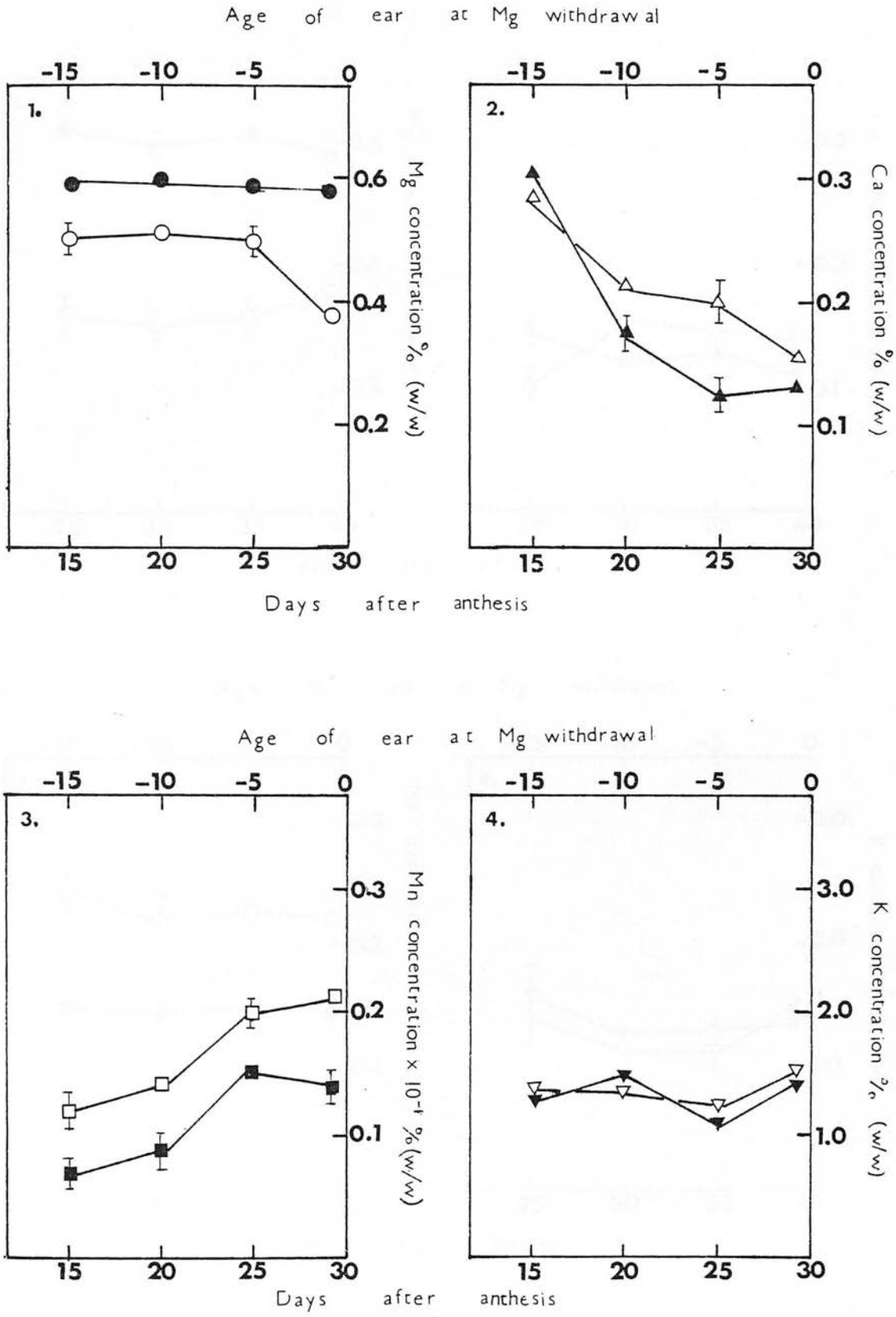


Fig. 7.6. Effect of variation in Mg supply on the concentration of Mg, Ca, Mn and K during embryo development. Samples harvested 30 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See facing page for legend).

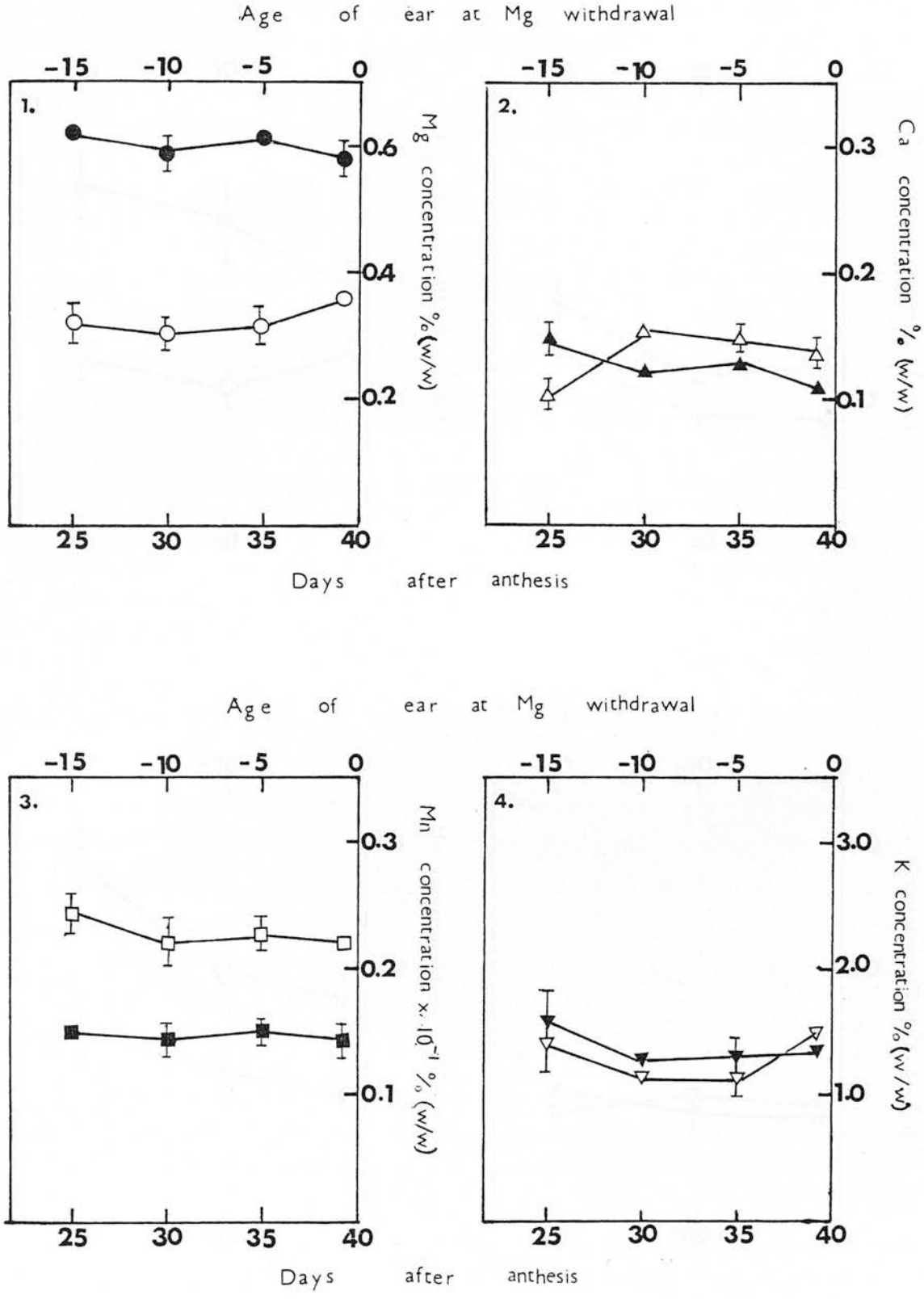


Fig. 7.7. Effect of variation in Mg supply on the concentrations of Mg, Ca, Mn and K during embryo development. Samples harvested 40 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See legend to Fig. 7.6.).

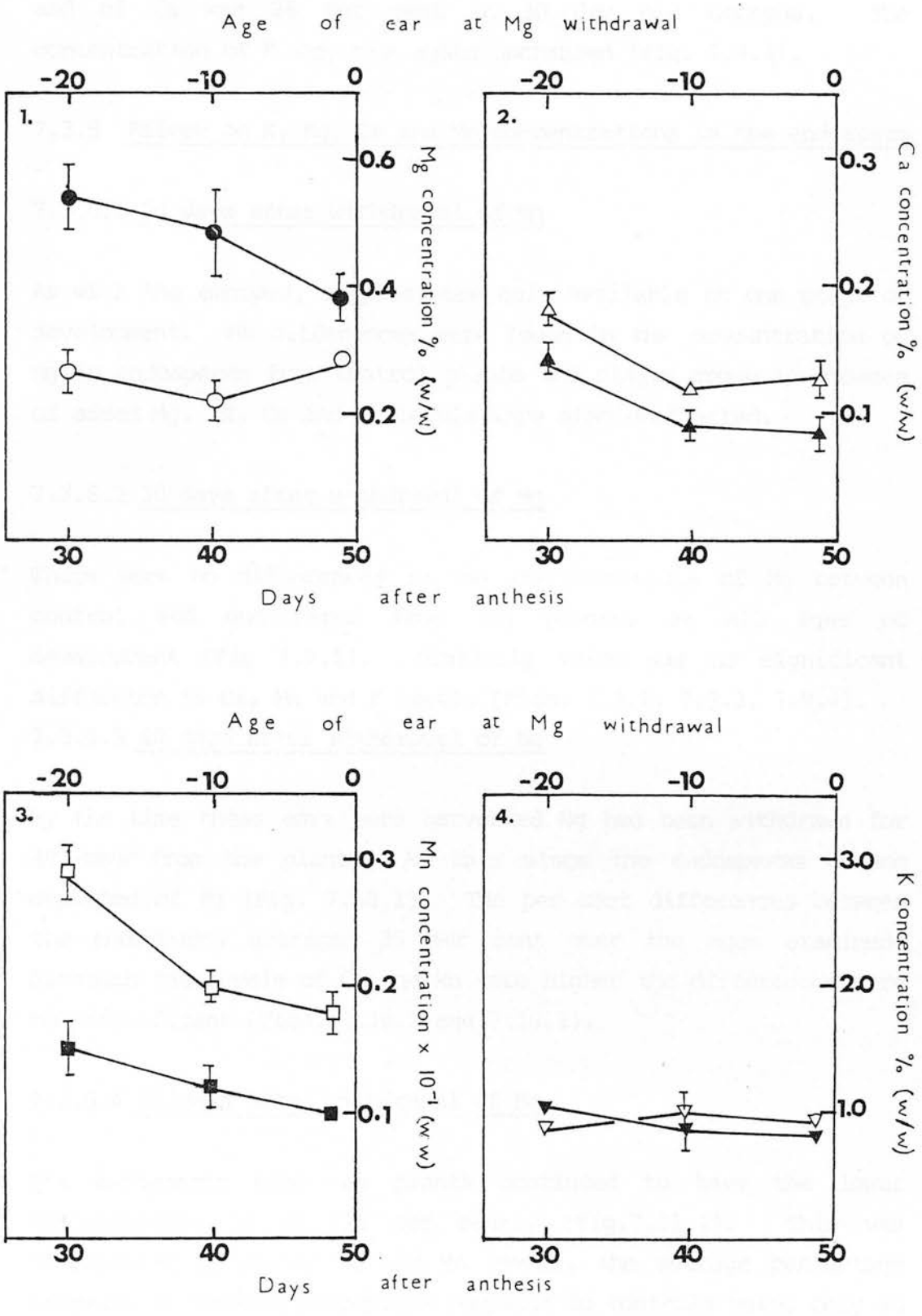


Fig. 7.8. Effect of variation in Mg supply on the concentrations of Mg, Ca, Mn and K during embryo development. Samples harvested 50 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See legend to Fig. 7.6.).

and of Ca was 26 per cent in 30 day old embryos. The concentration of K was once again unchanged (Fig. 7.8.4).

7.3.5 Effect on K, Mg, Ca and Mn concentrations in the endosperm

7.3.5.1 20 days after withdrawal of Mg

As with the embryos, samples were only available at one stage of development. No differences were found in the concentration of Mg in endosperms from control plants and plants grown in absence of added Mg. K, Ca and Mn levels were also unaffected.

7.3.5.2 30 days after withdrawal of Mg

There were no differences in the concentrations of Mg between control and endosperms from -Mg plants, at all ages of development (Fig 7.9.1). Similarly there was no significant difference in Ca, Mn and K levels (Figs. 7.9.2, 7.9.3, 7.9.4).

7.3.5.3 40 days after withdrawal of Mg

By the time these ears were harvested Mg had been withdrawn for 40 days from the plant. At this stage the endosperms became depleted of Mg (Fig. 7.10.1). The per cent differences between the endosperms averaged 25 per cent over the ages examined. Although the levels of Ca and Mn were higher the differences were not significant (Figs. 7.10.2 and 7.10.3).

7.3.5.4 50 days after withdrawal of Mg

The endosperms from -Mg plants continued to have the lower concentration of Mg (25 per cent), (Fig.7.11.1). This was accompanied by higher Ca and Mn levels, the average percentage increase in treated endosperms compared to controls being only 30 per cent for Ca and 40 per cent for Mn (Figs. 7.11.2 and 7.11.3). The K levels remained the same once again (Fig. 7.11.4).

Figs. 7.9., 7.10., & 7.11.

Variations in concentrations of 1. Mg (●—●), 2. Ca (▲—▲), 3. Mn (■—■) and 4. K (▼—▼) in developing endosperms from control plants (closed symbols) and from Mg withdrawn plants (open symbols).

The points represent means ± average errors from two experiments.

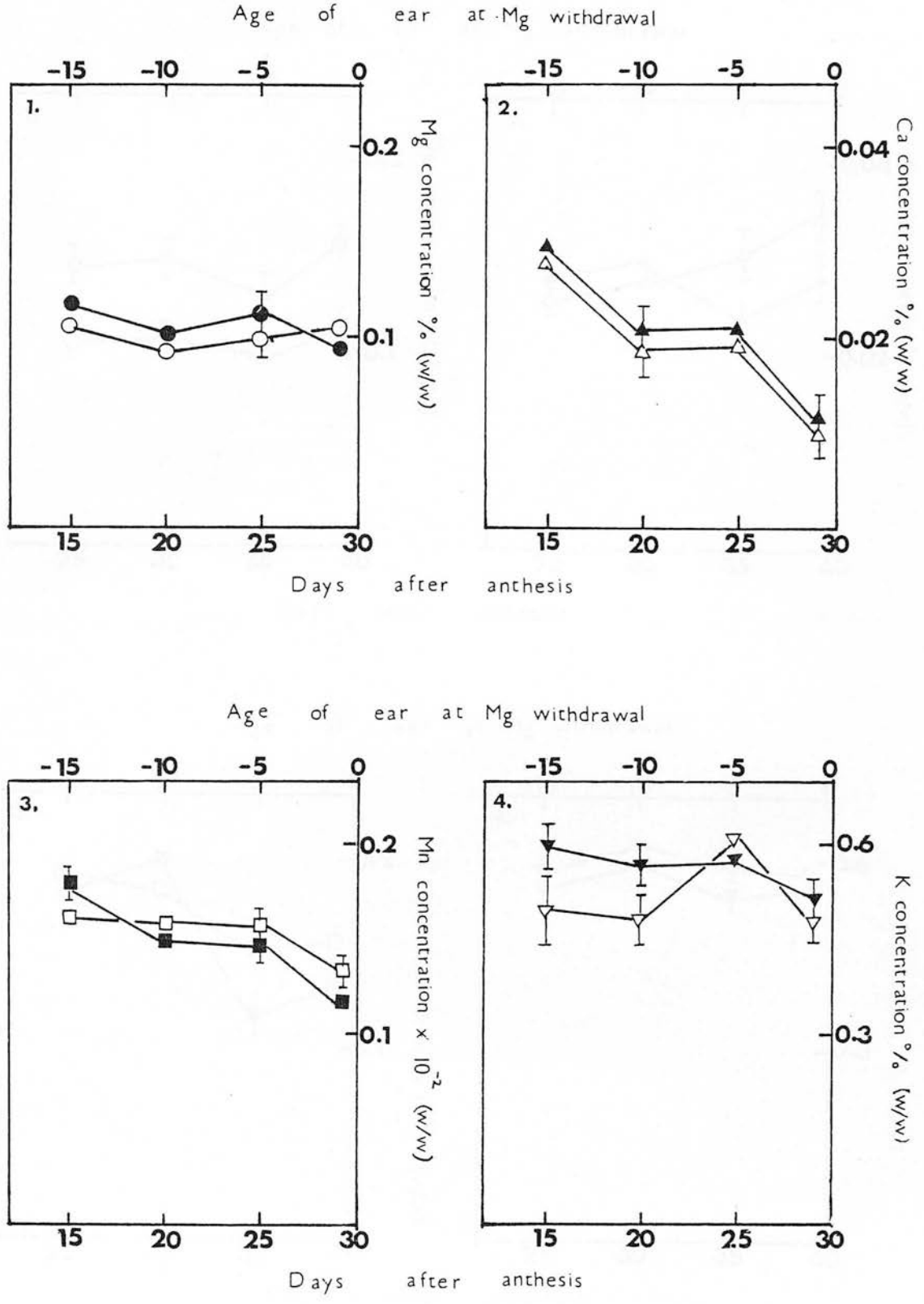


Fig. 7.9. Effect of variation in Mg supply on the concentrations of Mg, Ca, Mn, and K during endosperm development. Samples harvested 30 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See facing page for legend).

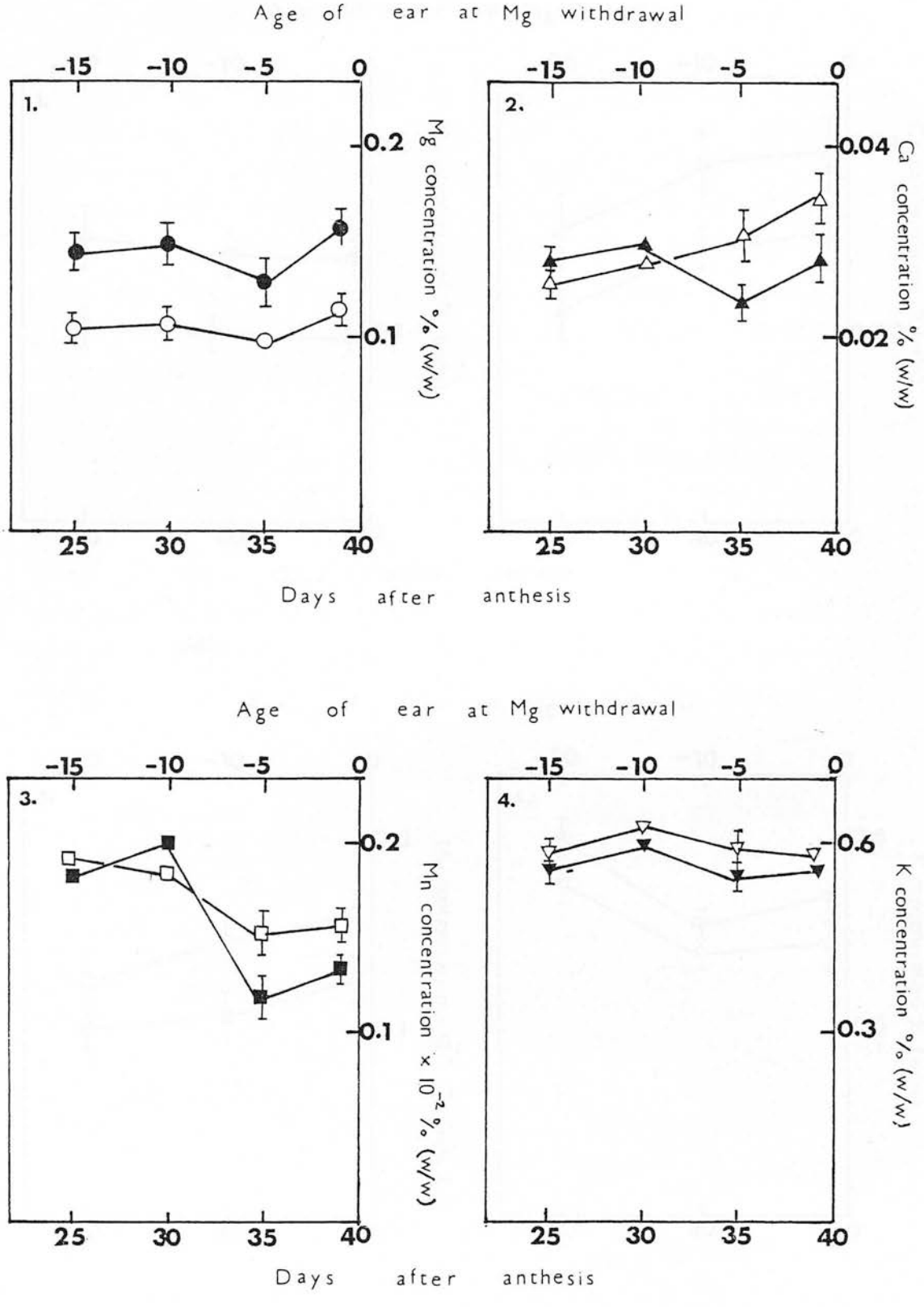


Fig. 7.10. Effect of variation in Mg supply on the concentrations of Mg, Ca, Mn and K during endosperm development. Samples harvested 40 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See legend to Fig. 7.9.).

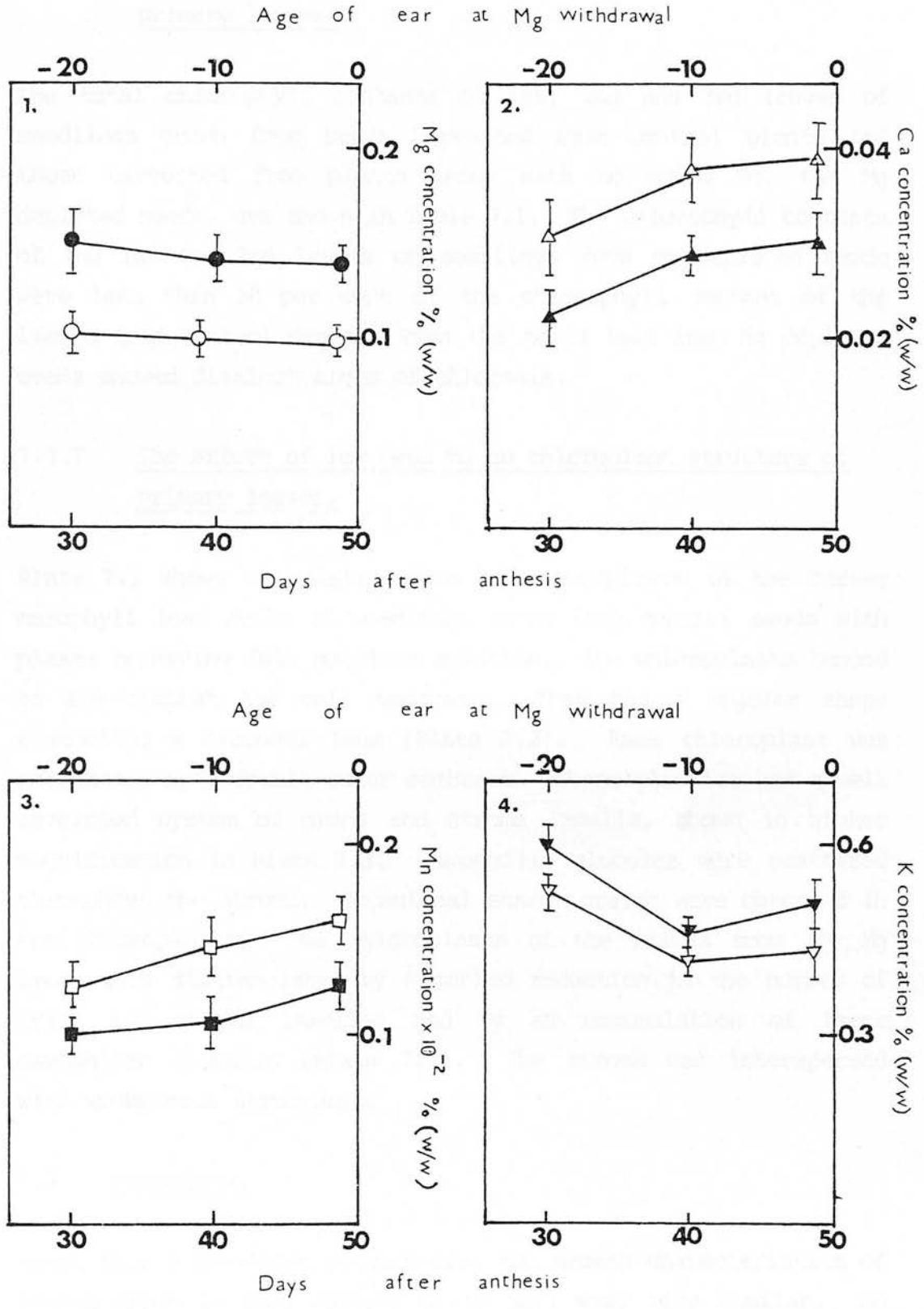


Fig. 7.11. Effect of variation in Mg supply on the concentrations of Mg, Ca, Mn and K during endosperm development. Samples harvested 50 days after Mg withdrawal from plants grown under conditions described for Fig. 7.3. (See legend to Fig. 7.9.).

7.3.6 The effect of low seed Mg, on chlorophyll content of primary leaves.

The total chlorophyll contents of 1st, 2nd and 3rd leaves of seedlings grown from seeds harvested from control plants and those harvested from plants grown with no added Mg, the Mg depleted seeds, are shown in Table 7.1. The chlorophyll contents of the 1st and 2nd leaves of seedlings from Mg depleted seeds were less than 30 per cent of the chlorophyll content of the leaves from control seeds. Even the third leaf from Mg depleted seeds showed distinct signs of chlorosis.

7.3.7 The effect of low seed Mg on chloroplast structure of primary leaves.

Plate 7.1 shows the distribution of chloroplasts in the barley mesophyll leaf cells of seedlings grown from control seeds with plants receiving full nutrient solution. The chloroplasts tended to lie against the cell membrane. They had a regular shape resembling a biconvex lens (Plate 7.2). Each chloroplast was surrounded by a double outer membrane. These plastids had a well developed system of grana and stroma lamella, shown in higher magnification in Plate 7.3. Osmophilic globules were scattered throughout the stroma. Occasional starch grains were observed in the chloroplasts. The chloroplasts of the leaves from low Mg seeds were distinguished by a marked reduction in the number of grana and stroma lamellae and by an accumulation of large osmophilic globules (Plate 7.4). The stroma was interspersed with membranous structures.

7.4 Discussion

Apart from a few minor differences, the growth characteristics of plants grown in sand culture or in soil were very similar. No significant effects on tiller numbers of the Mg treatments were

Table 7.1. Chlorophyll content of 1st, 2nd and 3rd leaves of seedlings grown from Mg depleted and control seeds. The seeds were sown in acid washed sand and no further nutrient solutions were applied. The values represent means ± standard deviations derived from triplicate determinations.

Leaf	Chlorophyll content mg Chlorophyll/mg Dry weight	
	Control	Magnesium deficient
1st	0.244 ±0.022	0.095 ±0.0341
2nd	0.270 ±0.0277	0.093 ±0.0100
3rd	0.233 ±0.0331	0.187 ±0.0270

The effect of low seed Mg on chloroplast structure of primary leaves.

Abbreviations

is = Intercellular space

g = Granum

og= Osmophilic globule

tm= Thylakoid membrane

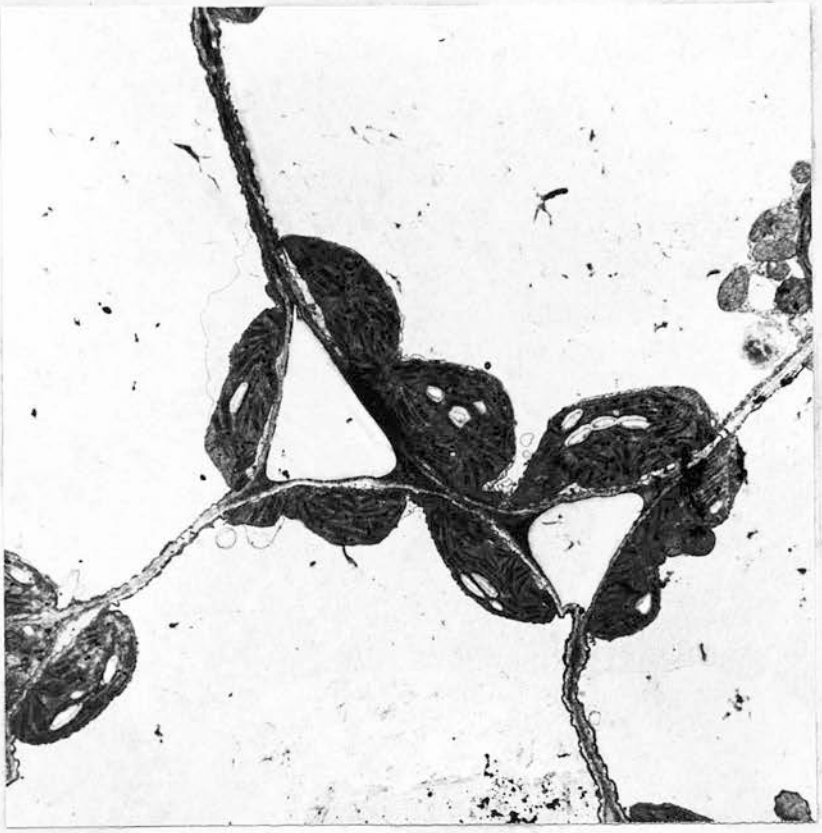
Plate 7.1 Distribution of chloroplasts in mesophyll leaf cells of barley seedlings cv. Midas, grown in sand culture. X 4,000.

Plate 7.2 Chloroplast in leaf mesophyll cell of barley seedlings cv. Midas, grown in sand culture from seeds containing adequate Mg. X 24,000.

Plate 7.3 Granum and osmophilic globules in leaf mesophyll cell of barley seedling cv. Midas, grown in sand culture from seeds containing adequate Mg. X150,000.

Plate 7.4 Thylakoid membranes and increased number of osmophilic globules in chloroplast of leaf mesophyll cell of barley seedling cv. Midas, grown in sand culture from seeds containing inadequate Mg. X42,000.

7.1



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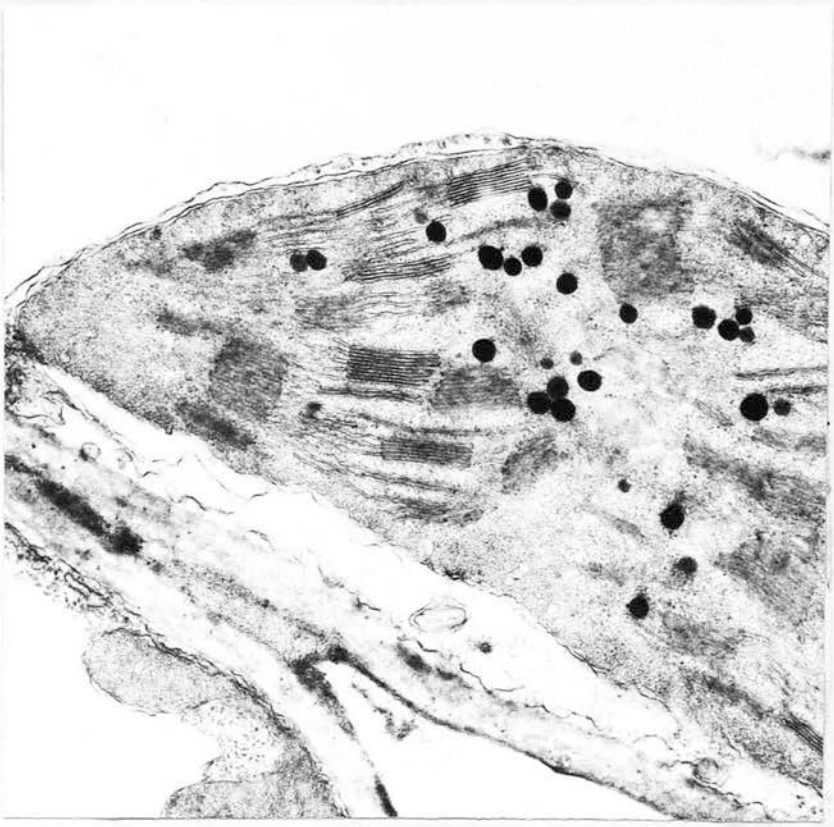

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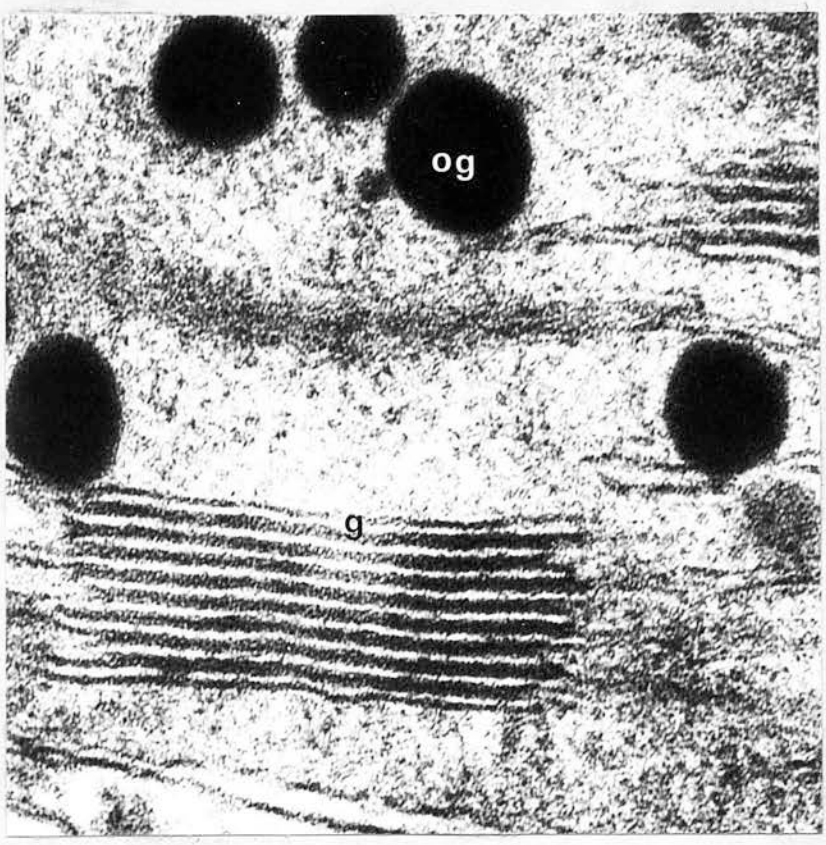

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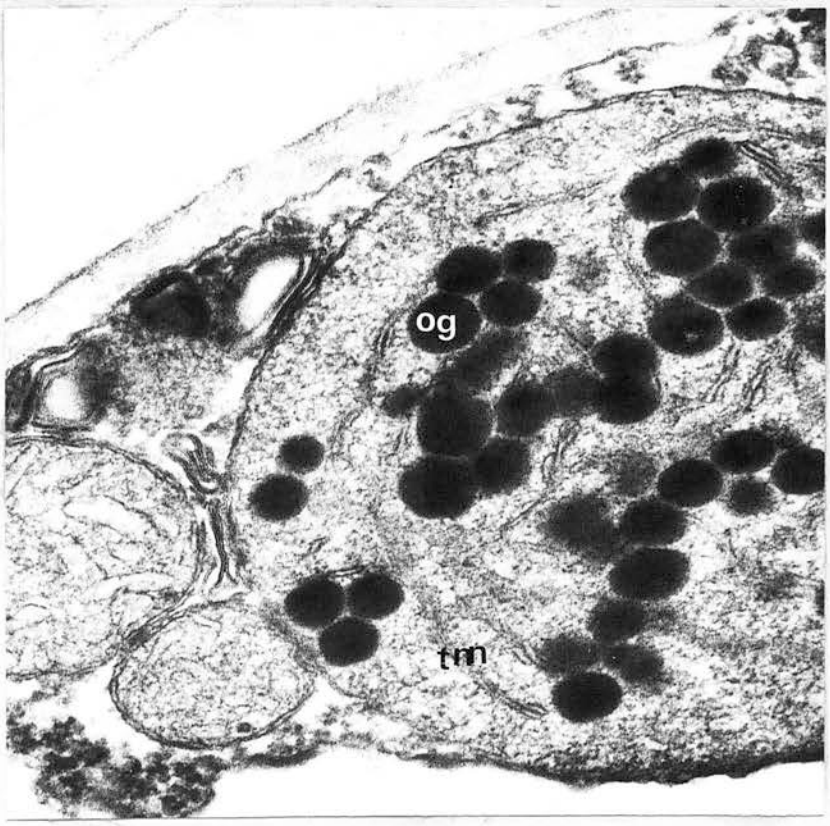


observed. Thus, either Mg levels were not critical in determining tiller numbers or tillering was encouraged by the continuous supply of other nutrients in defined solutions (sec.1.2). The latter is probably more likely since tiller number is considered to be a function of mineral nutrient supply (Aspinall 1961, Fletcher & Dale 1973).

The variation in weights between ears of corresponding ages harvested at different times during the experiment was probably due to differences occurring in grains from main ears, primary tillers and secondary tillers. The grains of both control and treated plants, sampled 30 days after Mg deprivation, were from the main ears and some primary tillers. Most of those sampled later in the experiment were secondary and lower order tillers. In addition to this by the time the last set of ears was harvested, the plants were dry and transpiration minimal as compared to the relatively healthy condition of plants earlier in the experiment. However the order at which the ears anthesed should not have influenced the results of Mg withdrawal since the ears at each age, both for control and -Mg were sampled at an identical stage of plant development. Although dry weights of e.g. control embryos and endosperms of any age varied between harvests, the concentrations (on a dry weight basis) of the elements examined remained constant and hence the values could be directly compared for the three periods of Mg withdrawal.

Embryos from ears of -Mg plants harvested 20 days after Mg withdrawal, showed a slight reduction in concentration of Mg; this being sufficient to induce an influx of Mn into the embryo. However, significant reductions of Mg (33 per cent w/w) which may be considered as 'deficient levels' were only observed in 30 day old ears.

In the early stages of development, mineral elements would be supplied to the developing grain both from the soil via the root system and from other plant parts by the internal redistribution of nutrients (sec. 1.7). These mechanisms normally ensure a supply of Mg to both the embryo and endosperm. In addition to the steady state redistribution due to the sink/source effect, an increased transport of some mineral elements from the leaves occurs when the exogenous supply of elements becomes limiting. For example when Zea mays plants were deprived of an external supply of N, P, K, Ca and Mg immediately after silking, an increased movement of all these, with the exception of Ca, into developing fruits was observed (Kissel and Ragland 1967). Further, when wheat plants were deprived of N in the rooting medium at anthesis, a greater migration of N from the leaves and stems to the ear was observed in the deprived plants compared to controls supplied with N throughout ear development (Neales *et al.* 1963). In fact a small, but significant increase in the N content of the grain at maturity in N deprived plants was observed. Hence, under conditions of Mg deprivation, there may be a greater movement of Mg out of the barley leaves and stem to the grain, than in those grown under conditions of optimal Mg supply.

The mobility of an element, within the plant can also affect its availability, regardless of its presence in the rooting medium. The mobility of Mg is intermediate between the more mobile N, P, and K and the less mobile Ca, Fe and Mn (Bukovac & Wittwer 1957, Ziegler 1975, see sec 1.8). Thus if external supplies are inadequate, Mg would be remobilised from stems and leaves to young growing tissues via the phloem. However, at and beyond 30 days of Mg deprivation the lower Mg levels observed in the embryo indirectly reflect the lower Mg status of the whole barley plant.

The lower Mg values not only persisted in the embryos harvested 40 and 50 days after Mg withdrawal, but there was a progressive drop in the levels measured. Transpiration of the barley plants was minimal at this stage and the amount of Mg redistributed was presumably low. However, about the time of harvests, the leaves began to senesce and release of inorganic ions from leaves due to an increase in the permeability of cell membranes may have been an added source of ions to the developing grain. During leaf senescence the content of P, N, K, Cl and Mg decreased noticeably (Pate 1975). Varying Mg concentrations in the rooting medium should not effect its remobilisation. However, only limited redistribution of Mg compared to N occurred during senescence in Anoka Soybeans (Derman *et al.* 1978). Results obtained by these workers were open to question, since K, an extremely mobile element (Sprague 1964) was apparently not redistributed.

Exactly how much of the nutrient loss, including inorganic ions from senescing organs actually reaches the grain is questionable. Raw materials in wheat used in endosperm and embryo growth moved up the vascular strand of the crease before entering the endosperm (Frazier and Appalanaidu 1965). At about 35-40 days after anthesis the cells of the pigment strand, those between the chalaza and the nucellar projection were observed to become impregnated with lipoid material. This material was suggested to block nutrient uptake into the grain and the onset of fall in fresh weight of wheat grain was attributed to this (Sofield *et al.* 1977). Thus, these results indicated that once the "lipid block" was set no further transport to or from the grain occurred.

The mature pericarp may also be a source of mineral ions for the embryo and the endosperm. Much of N, P, K and Mg located in the pericarp of the grain during development is lost by 40 days after anthesis. Corresponding increases have been observed in the endosperm and the embryo and a mechanism whereby direct transfer of nutrients from pericarp to the other two tissues has been postulated (Duffus & Rosie 1976a). Since Mg was not measured in

the pericarp during the course of the present experiment, the extent of its contribution to the endosperm and embryo is not known. However, the pericarp of Mg deficient plants may itself also have had lower Mg concentrations.

No difference in endosperm Mg levels between the treatments was observed until 40 days after Mg withdrawal. The Mg concentration in Mg - deprived endosperms was reduced by an average of 25 per cent (w/w), this being half as much as that observed between the embryos (50 per cent) at the same stage. Whilst a further reduction (60 per cent) was observed in the embryos in samples harvested 50 days after treatment application, the level in the endosperms (25 per cent) remained unaltered. Furthermore the embryos became deficient rather earlier in grain development than the endosperms. This may suggest that the inorganic nutrients entering the grain reach the endosperm first where the Mg complement required for the endosperm at a particular stage is taken up, leaving the remaining Mg to supply the embryo. The embryo would then tend to become deficient before the endosperm. Alternatively, the embryo may receive inorganic nutrients directly from the plant vascular system and not via the endosperm.

The endosperms of Mg - depleted plants also contained higher concentrations of Mn than control endosperms when they began to show lower levels of Mg. It is interesting to note that a decrease of 25 per cent in the concentration of Mg compared to the controls in both embryos and endosperms induced an increase of about 40 per cent in Mn concentrations. Studies of isolated enzyme systems from plant and animal tissues have indicated that Mn can replace Mg in a number of enzyme catalysed reactions, in particular phosphorylating and group transfer reactions e.g. glucokinase, phosphoglucomutase and phosphoglucokinase (Nason and McElroy 1963). Both the catalytic and allosteric regulatory properties of yeast phosphofructokinase were retained when Mg was replaced by Mn (Jones *et al.* 1972). However, in most cases where

Mn substitutes for Mg, the activity is somewhat lower. Although the per cent increase in Mn was much higher in the embryos with low Mg concentrations, than in the controls, these Mn concentrations may not be sufficient to abolish the Mg deficiency. Nevertheless, the two metal ions have much in common. For example the stability constants for ATP complexed with either Mg or Mn are of similar order in addition to the rate of formation of the ATP - metal ion complex (Mahler & Cordes 1971).

The present work suggests that it is likely that Mn can replace Mg in cytoplasmic processes since no significant differences were found in hexokinase activity between Mg - deficient and control embryos. Hexokinase is dependent on either Mg or Mn for activity (Dixon & Webb 1979) and these results suggest either that the low Mg levels present were sufficient enough for normal activity or that Mn levels in the Mg - deficient embryos enabled enzyme activity to express itself normally. It is always possible that the increased Mn uptake by Mg- deficient embryos may be a result of cation imbalance caused by removal of Mg.

Increased Mn absorption from the rooting medium by the plant probably met the demands of the Mg-deficient embryo and endosperm. Studies on the influence of Mg and Ca on Mn uptake by excised barley roots showed that Mg was highly inhibitory whilst Ca appeared to enhance the rate of Mn absorption (Maas *et al.* 1969). Mn had no effect on the Ca absorption but inhibited the absorption of Mg. It has been suggested that the selectivity in ion absorption resulted from cation-induced conformational changes in the structure of carrier molecules. In the present experiment, Mn sites could probably have become more available resulting in enhanced Mn uptake in the absence of Mg.

The increase in concentration of Ca in embryos containing lower Mg levels was probably due to a need for maintenance of cationic balance in the cells. A study of the interactions between cations in tomato, beans and maize plants showed that changes in the concentrations of Mg, Ca and K in test plants or their organs were mostly inversely related to changes in concentrations of one or two other cations (Radi *et al.* 1973).

In addition, increased uptake of Ca at lower levels of Mg may be due to less competition at the membrane sites. It has been suggested that the effects observed in numerous cell types in response to varying Ca concentration are produced by Ca competing with Mg for membrane sites (Rubin 1975).

The relative increase in Ca concentration was higher in embryos from ears harvested 30 days, rather than at 40 or 50 days, after Mg withdrawal. At the 30 day harvest stage when the plant is still transpiring, the increase in Ca levels could be attributed to xylem translocation. Beyond this, when transpiration is low and nutrient supply to the grain is dependent on internal redistribution via phloem, Ca translocation is very low. However, under some circumstances the mobility of Ca maybe altered. For exmple, Ca has been observed to enter the sieve tubes when Ca concentration in the region of the sieve tubes was high (Ringoet *et al.* 1967, 1968). Ca is then translocated in the lumen of the sieve tubes (Wiersum *et al.* 1971). The absence of Mg and Mn may have resulted in a higher uptake or remobilisation of Ca, hence making it available to the Mg deficient embryo.

The lower Mg levels observed in the developing seed parts i.e. the embryo and later the endosperm, resulted in chlorosis in the next generation seedlings, a well known symptom of Mg deficiency. As expected with such a mobile element, Mg deficiency was first seen in the older leaves.

Since no external source of nutrients was supplied to the germinating seeds, the nutrients arriving at the growing axis were limited. The Mg supply to the seedlings from -Mg seeds was presumably less than that in control seeds since not only did the mature embryo have lower Mg reserves than the control, but the endosperm, which acts as an additional source of raw materials including inorganic ions, for the rapidly growing seedlings, also had a lower Mg content. Hence although the mineral reserves of

control barley seeds supported healthy growth up to the five leaf stage beyond which the seedlings began to die back, the reserves in the treated (-Mg) seeds proved insufficient. Of course the possibility of airborne contamination as well as some from the sand can not be eliminated, but was unlikely.

The ultrastructure of chloroplasts from the chlorotic leaves was found to be abnormal. The osmophilic globules and membranous structures observed in the lumen are presumably remains of disintegrated grana. Such ultrastructural abnormalities are typical of those seen in chloroplasts from chlorotic leaves. Similar results were reported for Mg-deficient maize leaves which were characterized by an accumulation of large osmophilic globules and reduction in the amount of grana and stroma lamellae (Hall *et al.* 1972).

Although an abnormal accumulation of starch has been reported in chloroplasts of Mg-deficient bean (Whatley 1971) this was not observed in the present work.

Since the leaves were green and healthy initially and chlorosis only occurred when the germinating seed system became Mg deficient, the chloroplasts present in these leaves must initially have been completely developed and functioning normally. The disruption of chloroplast lamellar structure presumably occurred at the point where the seedlings became deficient in Mg, since relatively high cation concentrations have been reported to be required to maintain lamellar stacking (Smillie *et al.* 1976).

Since only 10 per cent of leaf Mg is associated with chlorophyll (Nason & McElroy 1963), there may be other lesions present such as reduced protein synthesis caused by impaired ribosome function and reduced activity of Mg-dependent enzymes.

In conclusion Mn appears to be able to compensate for Mg in some physiological functions during development, since the dry weights of embryos and endosperms were unaffected even though the Mg content was lower. However, where Mg is a structural component of a compound e.g. chlorophyll, Mn could not compensate for lack of Mg and this showed up as chlorosis in young seedlings.

The response of Mn and Ca to lower Mg levels was probably not osmotic, since K, which has an important osmotic role in the cell was unaffected. The developing grain as a whole appears to take up the additional Mn from the rooting medium to compensate for 'Mg deficiency' in the embryo. Whether or not the endosperm supplies the embryo with the additional Mn and in turn takes up additional Mn from the vascular system, is not clear. Indeed it did not appear to supply Mg to the embryo since not only did the embryo become 'Mg deficient' earlier but it also displayed a higher percentage of deficiency than the endosperm.

The longer the plant suffered from withdrawal of Mg, the greater was the 'deficiency' observed in the embryo. Once the Mg deposits in the plants, laid down before Mg withdrawal, had been exhausted through redistribution even the endosperm became 'Mg deficient'. A continuous supply of Mg to the plant during growth is hence necessary. Although the effects of inadequate supply are not obvious morphologically in the harvested seeds, the survival of the next generation seedlings may be endangered.

8.0 Effect of variation in the Mn supply on embryo and endosperm development during grain maturation *in vivo*.

8.1 Introduction

That Mn is an essential element for plant growth is well established. Its functions are related very closely to those of Mg (sec. 1.6.4). Mn deficiency is very common in cereals. It is aggravated under field conditions by overliming, and is common after cold wet weather which checks root growth. Some reports have shown that barley is more sensitive to Mn deficiency than wheat (Johansson & Ekman 1956, Stenuit & Piot 1957), others have suggested the reverse (Nyborg 1970).

If left untreated, Mn deficiency may seriously reduce yield. Agarwala *et al.* (1971) showed that grain yield in wheat was greatly reduced in plants grown under conditions of Mn deficiency and Mn levels in a number of different plant tissues were found to be much reduced.

The distribution of Mn in oat plants at different growth stages has been studied. The uptake of Mn continued throughout the whole period of growth but the relative rate of increase in the leaf was less after flowering (Williams & Moore 1952). The rate of uptake of Mn was also approximately equal to the rate of production of plant dry matter.

In the present investigation, plants were grown at two concentrations of Mn in the nutrient solution and the effect on embryo and endosperm weights studied. In addition, the effect of withdrawal of Mn at anthesis from some plants on the mineral nutrition of developing embryos and endosperms was studied.

8.2 Methods

Two sets of plants were grown in acid-washed sand (sec. 2.10.2) supplied with nutrient solutions containing 0.005mM and 0.002mM Mn. At anthesis Mn was withdrawn from one half of each set of plants (-Mn), the other half (control) continued to receive the same concentration of Mn up to maturity as before anthesis (sec. 2.10.2.2). The dry weights of embryos and endosperms of the two controls (0.005 and 0.002mM Mn) were compared. The changes in K, Mg and Ca, fresh and dry weight due to Mn withdrawal were investigated in embryos and endosperms at different stages of development, from ears that anthesed 15 days after Mn withdrawal. The mineral element concentrations in -Mn embryos and endosperms in both sets of plants, were expressed as percentages of control values.

8.3 Results

8.3.1 General Observations

No visual symptoms of Mn deficiency were observed in plants depleted of Mn at the concentrations used. Tiller counts carried out periodically during plant growth showed no differences between control and Mn depleted plants.

8.3.2 Effect on embryo and endosperm dry weight of Mn supply of 0.005mM and 0.002mM

Both embryos and endosperms from plants grown in 0.002mM Mn have significantly lower dry weights at later stages of development than those from plants with a Mn supply of 0.005mM (Fig. 8.1.1 and 8.1.2).

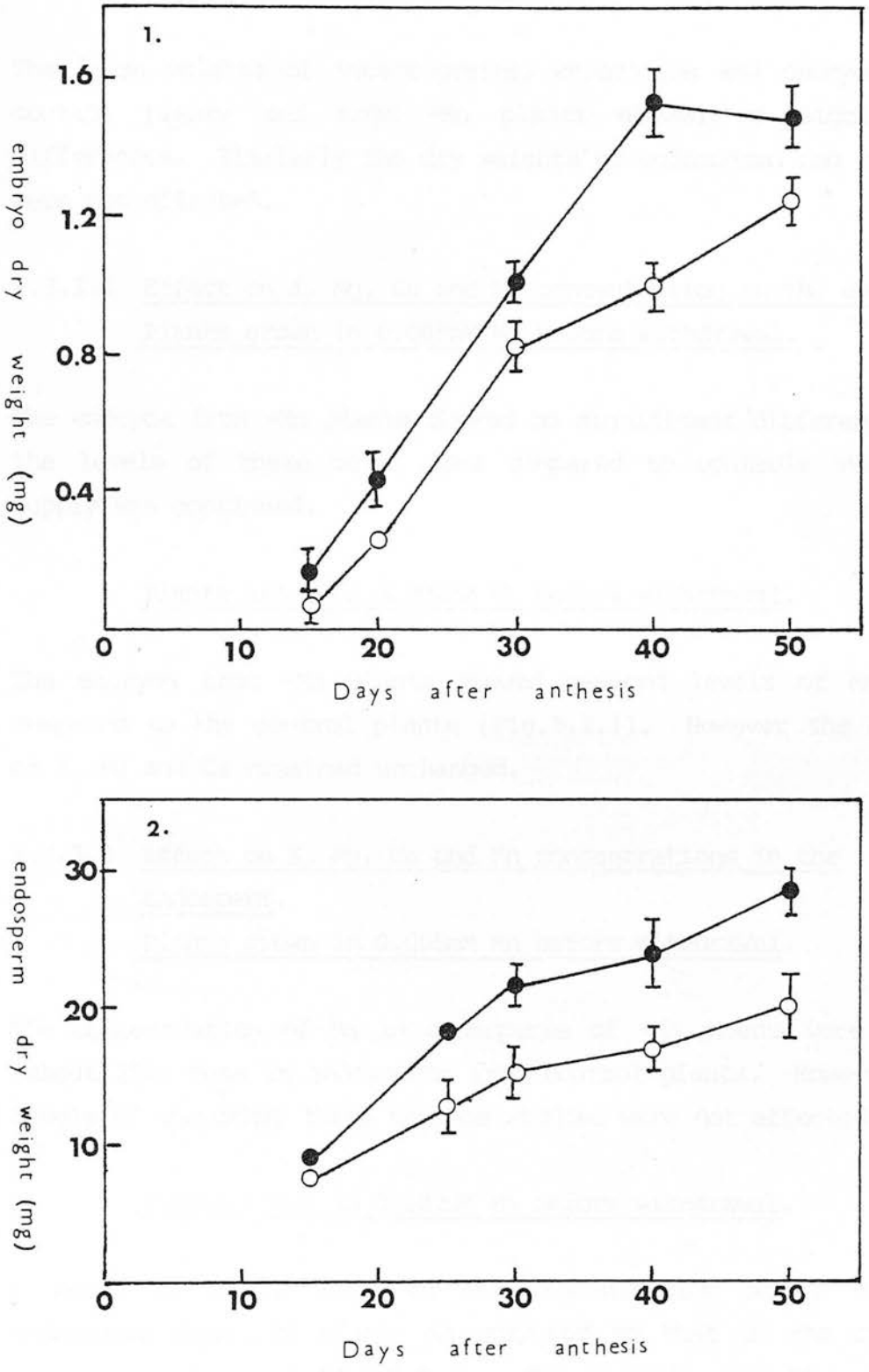


Fig. 8.1. The effect of variation in Mn supply on embryo and endosperm dry weight during development. Barley plants were grown in sand culture (sec.2.10) in Mn concentrations of 0.005 mM (closed symbols) and 0.002 mM (open symbols). The points represent means ± standard deviations of a minimum of triplicate samples.

8.3.3 Mn withdrawal

8.3.3.1 Effect on fresh and dry weights

The fresh weights of intact grains, endosperms and embryos from control plants and from -Mn plants showed no significant differences. Similarly the dry weights of endosperms and embryos were not affected.

8.3.3.2 Effect on K, Mg, Ca and Mn concentration in the embryo.

Plants grown in 0.005mM Mn before withdrawal.

The embryos from -Mn plants showed no significant differences in the levels of these metal ions compared to controls where Mn supply was continued.

Plants grown in 0.002mM Mn before withdrawal.

The embryos from -Mn plants showed reduced levels of Mn when compared to the control plants (Fig.8.2.1). However the levels of K, Mg and Ca remained unchanged.

8.3.3.3 Effect on K, Mg, Ca and Mn concentrations in the endosperm.

Plants grown in 0.005mM Mn before withdrawal.

The concentration of Mn in endosperms of -Mn plants were lower (about 25%) than in endosperms from control plants. However the levels of the other three cations studied were not affected.

Plants grown in 0.002mM Mn before withdrawal.

A reduction (about 20%) in the concentration of Mn in the endosperms from -Mn plants as compared to that in the control plants was observed (Fig.8.2.2). The decrease was higher than that in the corresponding embryos. Although consistently lower levels of Mg and higher levels of Ca were present in the treated endosperms compared to controls, the differences were not significant.

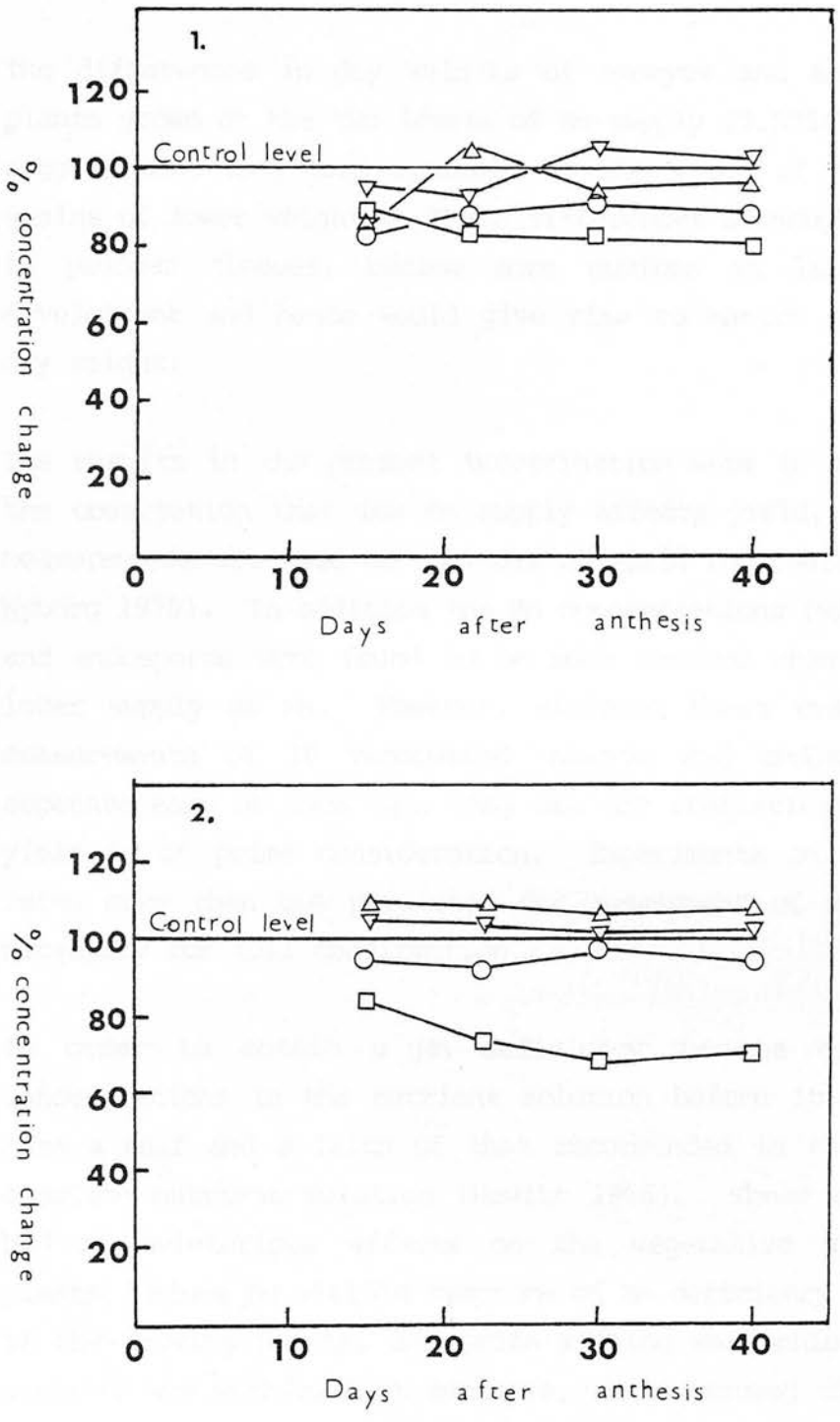


Fig. 8.2. Effect of variation in Mn supply on embryo and endosperm development. The percent changes in the concentrations of Mg (○—○), Ca (△—△), Mn (□—□) and K (▽—▽) in developing 1. embryos and 2. endosperms, harvested from plants where Mn was withdrawn compared to those from control plants. Plants were grown in sand culture in 0.002 mM Mn as described in sec.2.10. The points represent means from two experiments. The average errors were < 8 per cent.

8.4 Discussion

The differences in dry weights of embryos and endosperms from plants grown at the two levels of Mn supply (0.005mM and 0.002mM) suggest that long term exposure to low levels of Mn resulted in grains of lower weights. These differences although not apparent in younger tissues, became more obvious at later stages of development and hence would give rise to mature seeds of lower dry weight.

The results in the present investigation were in agreement with the observation that low Mn supply affects yield, if dry weight measurements are used as an index of yield (Agarwala *et al.* 1971, Nyborg 1970). In addition the Mn concentrations (w/w) of embryos and endosperms were found to be much reduced when grown in the lower supply of Mn. However, although these results involved measurements of 10 randomised embryos and endosperms from 3 separate ears at each age, they are not statistically valid when yield is of prime consideration. Experiments on a field scale using more than one perameter for assessment of yield would be necessary for full confirmation.

In order to obtain a Mn deficiency in the grain, the Mn concentrations in the nutrient solution before it was withdrawn were a half and a fifth of that recommended in the Long Ashton complete nutrient solution (Hewitt 1966). These concentrations had no deleterious effects on the vegetative growth of the plants. Since no visible symptoms of Mn deficiency were observed at the growing points, and grain filling was achieved in plants after Mn was withdrawn at anthesis, it is assumed that sufficient Mn was available either through transpiration or redistribution. The mobility of Mn in the phloem is dependent on the supply from the rooting medium. Under deficient conditions little or no redistribution within the wheat plant was observed, so that early leaves retained most or all of their Mn (Single 1958). The reduced levels of Mn in the endosperms in both experiments

indicate that the barley plants were becoming deficient in Mn. Any deficiency appearing in young growing regions is typical of a relatively immobile element since the older tissues retain the Mn and remain healthy (Sutcliffe & Baker 1976, Hewitt & Smith 1975).

Endosperms from -Mn plants of the set grown in 0.005mM Mn supply had lower Mn levels than the controls while the Mn contents of the embryo was unaffected. Hence it may be that the endosperm is capable of regulating the Mn supply to the embryo, thereby protecting it from damage. However, where the Mn supply from the rooting medium was insufficient (0.002mM Mn) the endosperm could not protect the embryo and both the embryo and endosperm had lower Mn contents than the control.

The developing maternal tissue of the grain, in particular the pericarp, may also have accounted in two ways for the reduced levels of Mn in the embryo. Firstly, the pericarp, which is green, and capable of photosynthesis (Nutbeam & Duffus 1978) would probably require Mn in higher amounts than the other tissues of the grain. Any Mn being redistributed through the phloem before the plant becomes adapted to Mn deficiency, may have been absorbed by the pericarp. Secondly, under conditions of reduced Mn supply from the rooting medium very little Mn would be translocated from the pericarp.

Although the grain tissues were just beginning to show Mn deficiency, similar effects were not observed for the other cations studied. A greater reduction in Mn levels in the grain tissues was probably required to create such a situation.

In conclusion, long term exposure to inadequate supplies of Mn lead to grains with lower dry weights, resulting possibly in lower yields. The endosperm appeared to supply Mn to the embryo and in turn itself became 'deficient', when Mn supply was withdrawn from the rooting medium. If however, the Mn content of the plants was lower before the Mn supply was withdrawn e.g. when they were grown in 0.002mM instead of 0.005mM Mn, the Mn supply to the endosperm was not sufficient to protect the embryo from a 'deficiency'.

9.0 Effect of variation in both the Mg and Mn supply on embryo and endosperm development during grain maturation *in vivo*.

9.1 Introduction

Since Mn can replace Mg and vice versa in a number of biochemical reactions, the removal of both from the rooting medium, might be expected to exaggerate the effects of the respective deficiencies. In addition, the redistribution of Mn and Mg would be affected by the supply of minerals in the rooting media. Hence the concentrations of these elements in the growing tissues i.e. embryos and endosperms would be affected.

The rate of nitrogen assimilation and conversion into nitrogenous compounds was affected by variation in the levels of both major and trace elements applied during plant growth (Hewitt 1963). Whether or not these effects extend to the harvested fruit or seeds is not known. In this experiment the effect of Mg and Mn depletion on the N status of the developing seed was also investigated using the micro-kjeldahl technique for N determination. The results obtained with this method are generally taken to represent the total N present, although in practice it does not measure nitrate or nitrite N. The effects on other mineral nutrients e.g. Ca and K in the developing grain, were also studied.

9.2 Methods

Plants were grown in acid-treated sand (sec. 2.10.2) and both Mg and Mn were withdrawn simultaneously at anthesis (sec. 2.10.2.3). The effects on both fresh and dry weights, and on concentrations (w/w) of K, Mg, Ca and Mn (sec.2.7)were evaluated at different stages of development in embryos and endosperms of ears that anthesed 15 days after main ear anthesis (2.10.4.3). The mineral element concentrations in -Mg-Mn embryos and endosperms were expressed as a percentage of control values.

Total endosperm N, of control and -Mg-Mn grains was measured by the micro-kjeldahl method (sec.2.10.6).

9.3 Results

9.3.1 Effect on fresh and dry weights

No significant differences were observed in the fresh and dry weights of both embryos and endosperms from control plants and -Mg-Mn plants.

9.3.2 Effect on K, Mg, Ca and Mn concentrations in embryos

Concentrations of Mg were considerably lower (average decrease 40 per cent) in embryos from -Mg-Mn plants than in the control plants (Fig.9.1.1). At the same time Mn and Ca concentrations of these embryos were higher than in the controls. The average increases in Mn and Ca over the developmental period when Mg concentrations were low, were 20% and 25% respectively. K levels were similar to those in control embryos.

9.3.3 Effect on K, Mg, Ca and Mn concentration in the endosperm

As with the embryos, the concentration of Mg in endosperms from -Mg-Mn plants was lower (average 50 per cent) than in the endosperms from control plants (Fig. 9.1.2). However, unlike the embryos, the concentration of Mn in these endosperms was also lower (average 30 per cent) than the control endosperms. The concentration of Ca remained unaltered, although a slight decrease in K levels in the endosperms from -Mg-Mn plants was observed.

The endosperms contained 30 per cent lower Mg than the control in 15 day old grains whilst the embryos showed a drop of only 10 per cent in these grains.

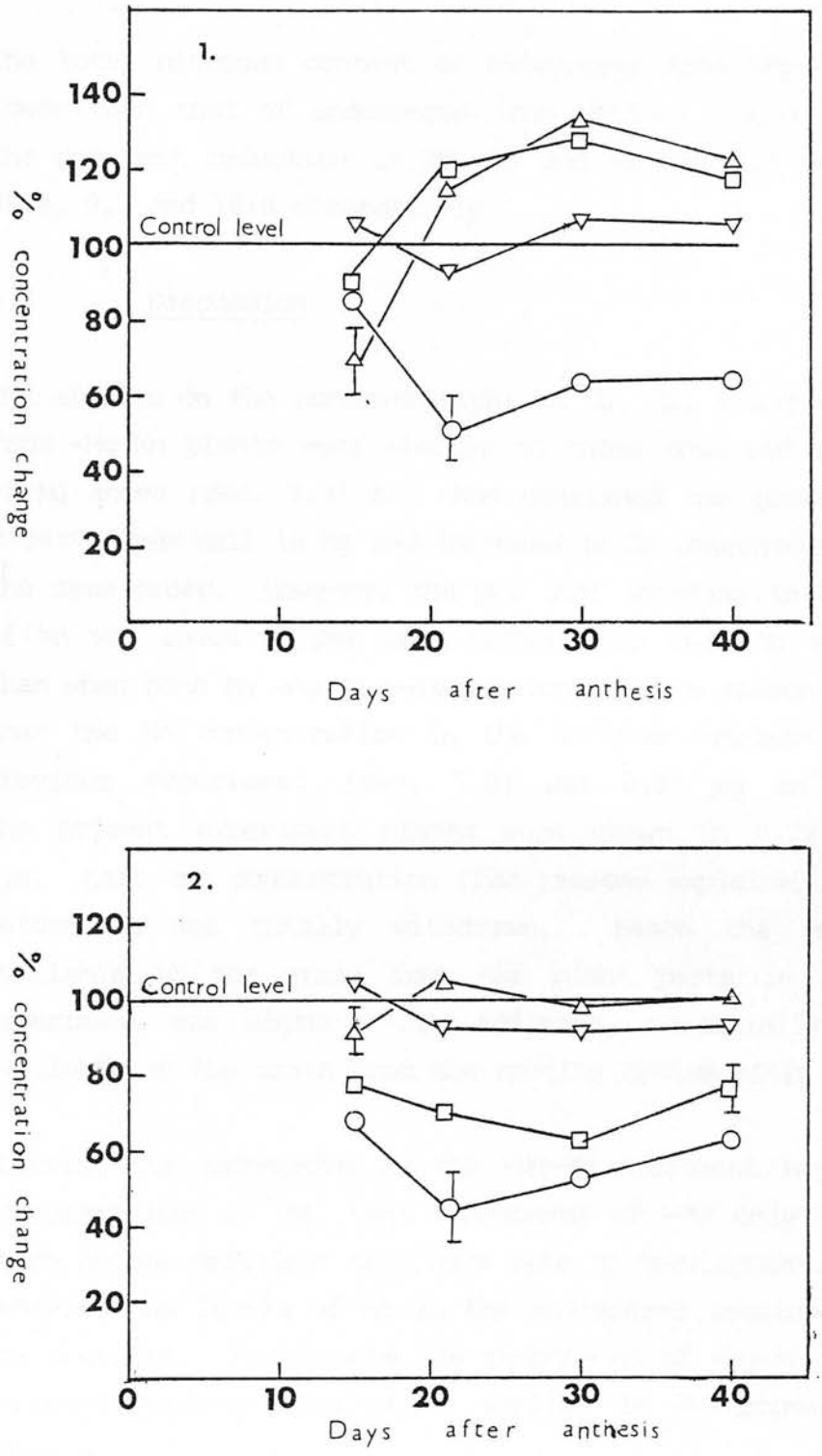


Fig. 9.1 Effect of variation in both the Mg and Mn supply on embryo and endosperm development. The percent changes in the concentrations of Mg (○—○), Ca (△—△), Mn (□—□) and K (▽—▽) in developing 1. embryos and 2. endosperms, harvested from plants where both Mg and Mn were withdrawan compared to those from control plants. Plants were grown in sand culture (sec 2.10.); Mg and Mn withdrawn as described in sec. 2.10.4.3. The points represent means ± average errors from two experiments. Unless indicated the average errors were < 5 per cent.

9.3.4 Effect on the total nitrogen content of endosperms

The total nitrogen content of endosperms from -Mg-Mn plants was lower than that of endosperms from control plants (Table 9.1). The per cent reduction in 25, 30 and 40 day old endosperms was 36.8, 9.7 and 16.8 respectively.

9.4 Discussion

The effects on the concentrations of Mg, Ca, K and Mn in embryos from -Mg-Mn plants were similar to those observed on withdrawal of Mg above (Sec. 7.3) and thus confirmed the previous results. The percentage fall in Mg and increase in Ca concentrations were of the same order. However, the per cent increase in concentration of Mn was about 50 per cent higher when only Mg was withdrawn than when both Mg and Mn were withdrawn. One reason for this was that the Mn concentration in the defined nutrient solution in previous experiment (sec. 7.0) was 0.55 $\mu g\ cm^{-3}$ whilst in the present experiment plants were grown in 0.28 $\mu g\ cm^{-3}$ Mn i.e. half the concentration (for reasons explained in sec. 8.4) before Mn was totally withdrawn. Hence the amount of Mn available to the grain from the plant parts in the previous experiment was higher. In addition, essentially no Mn was available to the grain from the rooting medium after anthesis.

However, the endosperms in the -Mg-Mn treatment had much lower concentrations of Mg, than endosperms of -Mg only (sec. 7.3.5) which became deficient only very late in development. Unlike the embryos, the levels of Mn in the endosperms remained lower than the controls. Furthermore the endosperms of -Mg-Mn plants had a depleted concentration of Mg earlier in development than the embryos.

In the absence, or limited supply, of Mn, the endosperm accumulated lower amounts of Mg than in the presence of Mn (sec. 7.3). This suggests that Mn may influence the Mg uptake mechanism in the endosperm. Alternatively insufficient Mg may be

Table 9.1: The total nitrogen content of endosperms during development from seeds where Mg and Mn were withdrawn (-Mg-Mn) and control seeds. The values represent a mean ± standard deviation derived from triplicate determinations.

AGE	Total nitrogen mg N/g Dry wt	
	Control	-Mg -Mn
25	31.2 ±3.5	19.7 ±3.3
30	33.1 ±2.7	29.9 ±0.6
40	34.0 ±0.7	28.3 ±1.2

translocated to the developing grain, the amount being less than that translocated at the same stage during Mg withdrawal. If the transport of mineral ions into the endosperm from the 'crease' region is energy-dependent (Fisher & Hodges 1969) then the ATPases required for Mg uptake may require specific concentrations of Mn for optimal activity.

Under the conditions reported here, where there was no external supply of Mg and Mn, the endosperm appeared to act as a source of Mn to the Mg-'deficient' embryo. The supply of Mn to the developing grain was greatly decreased due to reduction in the Mn supply via transpiration as well as a restricted redistribution from other plant parts, Mn redistribution being affected by the availability of Mn from the rooting medium (Single 1958). In addition, very little Mn may be translocated out from the pericarp (Duffus & Rosie 1976b). Hence the embryo appeared to be protected by the endosperm.

Angular brown flecks which develop between the veins of leaves are typical symptoms of Mn deficiency in barley (Hewitt 1963). The withdrawal of Mn at anthesis did not produce these symptoms indicating that the treatment did not induce a Mn deficiency in the whole plant. This was confirmed by the observation that some Mn was undoubtedly redistributed to the developing endosperm and embryo.

From the observations above there appeared to be an endosperm-embryo inter-relationship based on nutrient transfer during development. The relationship became obvious during stress conditions, where, if the supply of nutrients to the embryo was limiting, a mechanism may have been developed whereby the endosperm protected the embryo against any deficiency. This relationship is analogous to a source-sink phenomenon, and is well established in germinating seeds for organic and inorganic reserve materials. However, the concept that the embryo is dependent upon the endosperm for nourishment during development is based principally on surmise from indirect evidence (Raghavan 1976).

Alternatively, the Mn influx into the embryo when Mg concentrations were low, could have come directly from the vascular strands of the crease. Low concentrations of Mn in the endosperm may of course have been due to impairment of the Mn uptake mechanism to the endosperm as a result of Mg and Mn withdrawal. However, since there is no evidence for differential uptake mechanisms within the grain tissues, this may not be true.

The increase in embryo Ca is surprising because of its reported immobility in the phloem (sec.1.8). The Ca levels in the endosperm remained unaltered, irrespective of Mg levels.

The low nitrogen levels (dry weight basis) observed in the endosperms from plants grown under -Mg-Mn treatment, corresponded to low Mg and Mn levels in the endosperms. A major function of both Mg and Mn in biological systems is to maintain the integrity of the ribosome during protein synthesis (Hewitt 1963, Lyttleton 1960). Thus it may be that endosperm protein synthesis had been reduced, associated with a reduced amino acid flux into the endosperm. Of course not all of the N measured is protein and the function of Mg in a range of biological processes may simply have resulted in an overall decrease in endosperm N content.

In conclusion, the withdrawal of Mg and Mn caused a Mg 'deficiency' in both the embryo and the endosperm. However, in this case the 'deficiency' in the embryo was associated with an increase in embryo Mn content at the expense of the endosperm. The endosperm could not in turn replenish its Mn contents from the vascular system. Hence, the endosperm appears to supply Mn and possibly other elements to the developing embryo. The Mg and Mn 'deficiencies' in the endosperm resulted in a lower total nitrogen content, probably affecting the synthesis of protein. Due to insufficient material, it was not possible to test whether the increased Mn content in the embryo overcame the effect of Mg deficiency on the nitrogen content of the embryo.

10.0 *In vitro* barley embryo culture.

10.1 Introduction

It has not so far proved possible to grow large numbers of excised immature embryos in culture such that their development, growth and subsequent germination consistently resembles the *in vivo* process. The small size of these embryos and hence difficulty in removing them without damage from the maternal tissue has been one of the reasons for this. Only in recent years has particular emphasis been placed on the successful culture of cereal embryos. This is largely as a result of new developments in plant breeding (Kruse 1969, Kasha & Kao 1970) since the incompatibility which develops between embryo and endosperm in seeds derived from many hybrid crosses, necessitates their isolation and culture in order to ensure seedling formation.

Merry (1941, 1942) first made detailed comparisons of growth of barley embryos *in vitro* and *in vivo* in which embryos of varying sizes were grown on a solid agar medium based on Shrive's solution RSS2 containing 2 per cent sucrose. The results reported were similar to those of La Rue & Avery (1938) for wild rice. Embryos 0.75mm long grew and germinated, but the growth patterns were abnormal and precocious germination was prevalent. Embryos 0.35 - 0.60mm long displayed only limited growth and those smaller than 0.35mm failed to grow at all. Thus it appears. that *in vitro* culture was not possible until after a critical developmental stage.

Further studies by Chang (1963b) on barley embryos revealed that embryos 0.4mm long required 10.5 days to reach maturity (3.0mm long) *in vivo*; but a maximum length of only 1.8mm was obtained after 30 days in *in vitro* culture and the embryos were not fully developed. Therefore, in studies of barley embryogenesis in

culture, the main problem has been to obtain continuing embryo growth and development. Growth supplements such as coconut milk, casein hydrolysate and endosperm extracts have been added to culture media to improve survival of the barley embryos (see sec.1.11).

The use of these tissue extracts was found to be unsatisfactory and due to their undefined nature, the effects on growth could not be attributed to any one constituent. In this context Norstog & Smith (1963) attempted to devise a satisfactory synthetic medium of specified composition based in part on the analysis of coconut milk by Tulecke *et al.* (1961). The medium (Norstog Medium I) comprised a phosphate-enriched White's mineral medium, selected amino acids including glutamine and alanine, vitamins, malic acid and sucrose. The increased inorganic phosphate concentration in combination with the presence of glutamine and alanine improved both growth and differentiation of embryos 0.3 to 0.4mm long. Growth was assessed in terms of maximum length achieved in culture (5.2mm) compared to that of normal mature embryos (3.2mm), grown under *in vivo* conditions. Longitudinal sections revealed that the largest embryos in culture had developed four leaf primordia and five roots as compared to three leaf primordia and five roots in mature embryos grown *in vivo*. Hence, although the medium supported growth and differentiation, 'normal' development was not obtained. In addition no indication was given of the number of embryos cultured or the percentage success rate.

Problems were encountered with Norstog Medium I in later experiments when poor reproduceability was observed as well as a low percentage of embryo survival. A new medium (Norstog Medium II) appeared to be capable of supporting development of immature barley embryos and a 10 fold increase in embryo survival was reported (Norstog 1973). Before this, the survival rate in cultured barley embryos varied from 0 to 10. The changes from Norstog Medium I included increased K, and malic acid concentration and the elimination of niacin.

A survey of various published barley embryo culture media indicated that many of the media were unreliable and not likely to be of any value, particularly in large scale breeding programmes (Cameron-Mills & Duffus 1977). No parameters other than length were used to assess normality of *in vitro* cultured embryos e.g. morphological changes, fresh and dry weights, protein or carbohydrate contents. Although the embryo size values were an average of ten results, no indication was once again given of the frequency of survival of the cultured embryos. Even on this medium (Norstog II), embryos less than 0.2mm long failed to develop.

Since the greatest success with cultured barley embryos has been with Norstog Medium II, this was used in the present work to culture barley embryos cv.Midas. Growth characteristics using Bacto and Purified agar were observed, since both types have been used previously (Cameron-Mills & Duffus 1977, Norstog 1973) without comment on any differences in embryo growth between the two. The role of sucrose as an osmoticum was studied in relation to precocious germination.

Since few attempts have been made to assess the 'normality' of growth in cultured embryos by more than one - or at most two parameters - the present work has used a wide range of properties to see how far growth in culture resembles that *in vitro*. These include external morphology, germination characteristics, ultrastructure and dry weight measurements.

Since *in vitro* embryo culture as a technique lends itself ideally to the study of growth under defined conditions, it was used to investigate the effects of variation in the mineral ion supply particularly Mg on embryo growth and differentiation.

10.2 Methods

Embryos were dissected and cultured as described in secs. 2.11.1 - 2.11.4, on Bacto and Purified agar. Embryos were grown on media containing 0.1M and 0.2M sucrose in the dark before

germination, in order to assess the effects of sucrose concentration on growth (sec. 2.11.6). The growth of embryos was monitored by size measurements (sec. 2.4). Photographic (sec. 2.11.5), ultrastructural (sec. 2.12.1) and dry weight (sec. 2.5) comparisons between growth *in vitro* and *in vivo* were made using embryos of corresponding sizes grown *in vivo* under greenhouse conditions (sec. 2.2). Embryos were grown on a medium containing no added Mg as described in sec. 2.11.7.

10.3 Results

10.3.1 *In vitro* embryo growth

10.3.1.1 Culture of immature embryos on Bactoagar

Fig. 10.1 shows the growth of 0.55mm, 0.75mm and 0.83mm long embryos on Norstog's medium II solidified with Difco Bactoagar. In this experiment size (l x w) was used as an index of growth.

The bigger embryos ($0.50mm^2$) reached a size of $8.40mm^2$ after 7 days of culture, the $0.33mm^2$ embryos after 8 days and $0.22mm^2$ between 8 - 10 days. Hence embryos approximately 15, 14 and 12 days old (0.83mm, 0.73mm, 0.55mm long), when excised, grew to maturity after 7, 8 and 10 days in culture.

10.3.1.2 Culture of immature embryos on Purified agar

The increase in embryo size with time, of embryos 0.45mm, 0.55mm, 0.75mm and 0.83mm in length cultured on Difco Purified agar, is shown in Fig.10.2. The $0.48mm^2$ (0.83mm x 0.58mm) embryos reached a size of $8.0mm^2$ after 11 days, the $0.38mm^2$ after 12 days, the $0.22mm^2$ after 14 days, and the $0.11mm^2$ after about 16 days. The size measurements are an average of a minimum of ten determination from each of three separate experiments. Hence embryos approximately 15, 14, 13 and 10 days old at excision grew to maturity after 11, 12, 14 and 16 days respectively.

Fig. 10.1 Midas embryos of three sizes were grown on Norstog's Medium II containing 6 per cent Difco Bactoagar and 0.2M sucrose.

Fig. 10.2 Midas embryos of four sizes were grown on Norstog's Medium II containing 6 per cent Difco Purified agar and 0.2M sucrose.

The measurements are means of a minimum of twenty determinations with standard deviations about the means. Unless indicated all the standard deviations were <6 per cent about the mean. Embryo dimensions (length x width) are represented adjacent to each graph line.

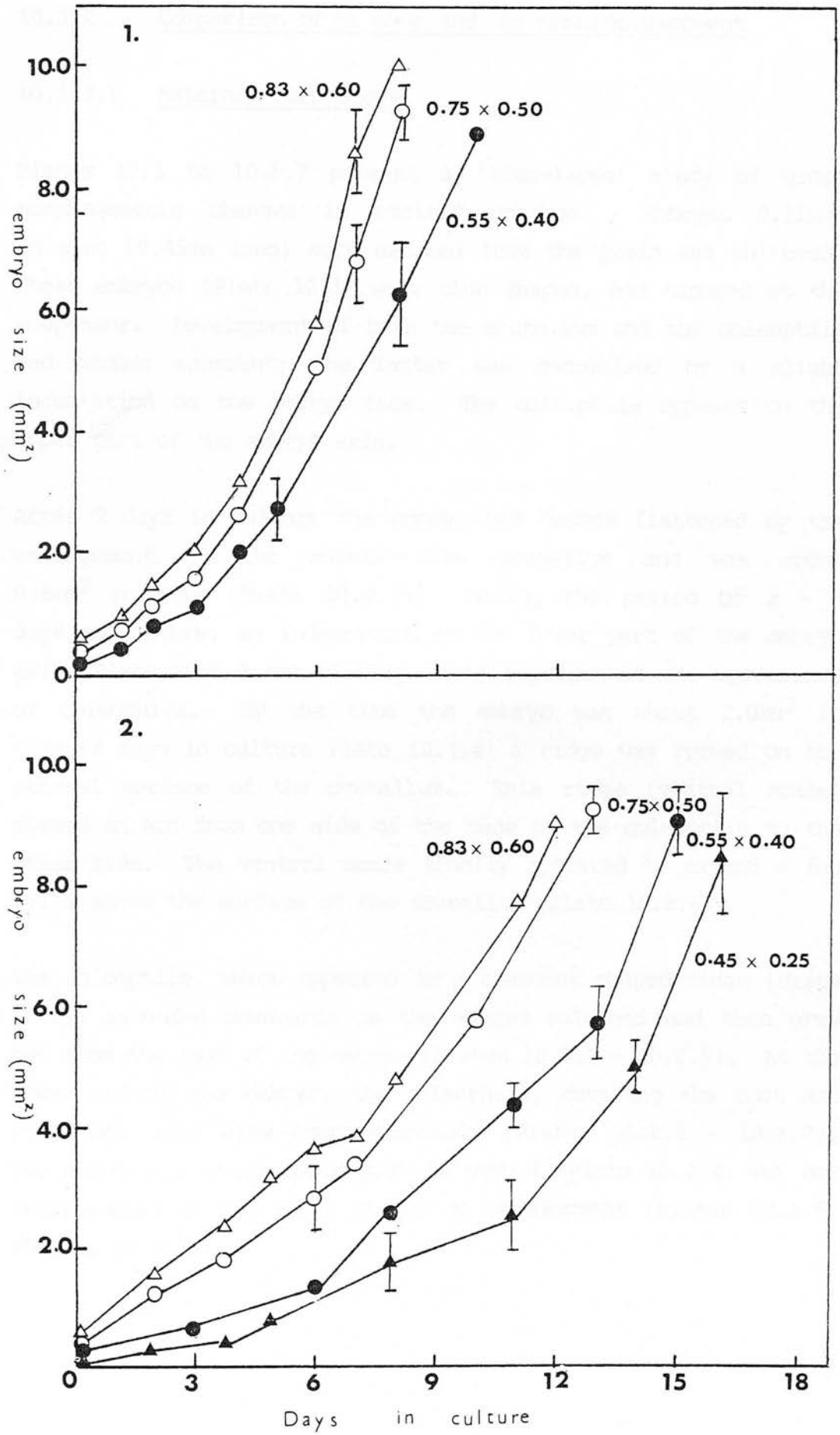


Fig. 10 . The growth of small barley cv. Midas embryos in vitro. (see facing page for legend).

10.3.2 Comparison of *in vivo* and *in vitro* development

10.3.2.1 External morphology

Plates 10.1 to 10.2.7 present a 'time-lapse' study of gross morphogenetic changes in excised embryos. Embryos 0.11mm^2 in size (0.45mm long) were excised from the grain and cultured. These embryos (Plate 10.1) were club shaped, and tapered at the suspensor. Development of both the scutellum and the coleoptile had become apparent; the latter was recognised by a slight indentation on the embryo face. The coleoptile appears on the upper part of the embryo axis.

After 2 days in culture the embryo had become flattened by the enlargement of the shield - like scutellum and was about 0.8mm^2 in size (Plate 10.2.2). During the period of 2 - 5 days in culture, an indentation at the lower part of the embryo axis (Plate 10.2.3 not clearly seen) represented the appearance of coleorhiza. By the time the embryo was about 2.0mm^2 in size (8 days in culture Plate 10.2.4) a ridge was formed on the ventral surface of the scutellum. This ridge (ventral scale) formed an arc from one side of the base of the coleoptile to the other side. The ventral scale finally appeared to extend a few cells above the surface of the scutellum (Plate 10.2.6).

The coleoptile, which appeared as a crescent shaped ridge (Plate 10.1), extended downwards as the embryo enlarged and then grew out from the rest of the embryo (Plates 10.2.5 - 10.2.7). At the lower end of the embryo, the coleorhiza, covering the root and root cap, also grew proportionately (Plates 10.2.3 - 10.2.7). The suspensor, which could just be seen in Plate 10.2.4, was not recognisable at the later stages of development (Plates 10.2.5, 10.2.6, 10.2.7).

Comparison of *in vivo* and *in vitro* embryo development: External Morphology. Excised embryos of barley cv. Midas were cultured *in vitro* on Norstog's Medium II (0.2M sucrose, .Purified agar). Embryos of corresponding sizes were dissected from grains harvested from plants grown *in vivo* under greenhouse conditions.

Plate 10.1 Embryo 0.11 mm^2 in size was placed on culture medium. X400

Plate 10.1.2 Embryo 0.8 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.2 Embryo 1.0 mm^2 in size, 3 days after culture *in vitro*. X 400.

Plate 10.1.3 Embryo 1.6 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.3 Embryo 1.4 mm^2 in size, 5 days after culture *in vitro*. X 400.

Plate 10.1.4 Embryo 1.9 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.4 Embryo 2.1 mm^2 in size, 9 days after culture *in vitro*. X 400.

Plate 10.1.5 Embryo 2.6 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.5 Embryo 2.6 mm^2 in size, 11 days after culture *in vitro*. X 400.

Plate 10.1.6 Embryo 4.2 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.6 Embryo 4.5 mm^2 in size, 13 days after culture *in vitro*. X 400.

Plate 10.1.7 Embryo 8.8 mm^2 in size from plants grown *in vivo*. X 400.

Plate 10.2.7 Embryo 8.5 mm^2 in size, 16 days after culture *in vitro*. X 400.

10.1

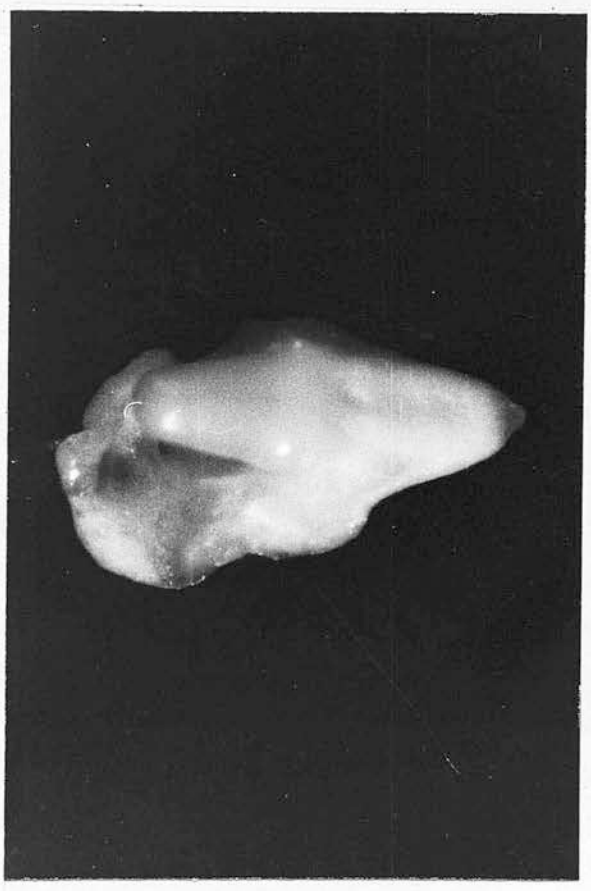
10.1.2

10.2.2

10.1.3

10.2.3

10.1.7



10.2.7

Plates 10.1 - 10.1.7 are a photographic representation of embryos of equivalent sizes excised from grains harvested from plants grown *in vivo* . The external morphological changes were similar to those described above for *in vitro* development. The shape of the scutellum after a few days in culture (Plates 10.2.4-10.2.7) deviated from the shape of *in vivo* grown embryos. At maturity, the scutellum began to curl up (Plate 10.2.7).

10.3.2.2 Germination

The germination characteristics of embryos grown under *in vitro* culture conditions (Norstog II) were compared with those of embryos excised from a 35 day old grain (embryo size 8.5mm^2). The basis of the comparison used was shoot and root growth as estimated by coleoptile length, 1st leaf length, number of roots and total length of the roots (Fig.10.3). In general there was no significant difference between the two, although those embryos grown *in vitro* tended to have smaller numbers of roots. Although root numbers were smaller in seedlings from embryos grown *in vitro* , the total root length was not significantly different.

10.3.2.3 Dry weights

The dry weights of embryos grown *in vitro* were not significantly different from those grown *in vivo* (Fig.10.4).

10.3.2.4 Ultrastructure

At the stage of development investigated (7.0mm^2 in size) the embryos grown both *in vitro* and *in vivo* were observed to contain mitochondria with many cristae (Plates 10.3.1, 10.3.2). Numerous ribosomes present as polysomes and extensive rough endoplasmic reticulum were observed in both types of embryo cells (Plates 10.3.1, 10.3.2, 10.3.3, 10.3.4). Accumulation of storage material was prevalent in *in vitro* as well as *in vivo* grown embryos. Amyloplasts with starch grains can be seen in Plates 10.3.5, 10.3.2. Plate 10.3.6 shows pro-plastids,

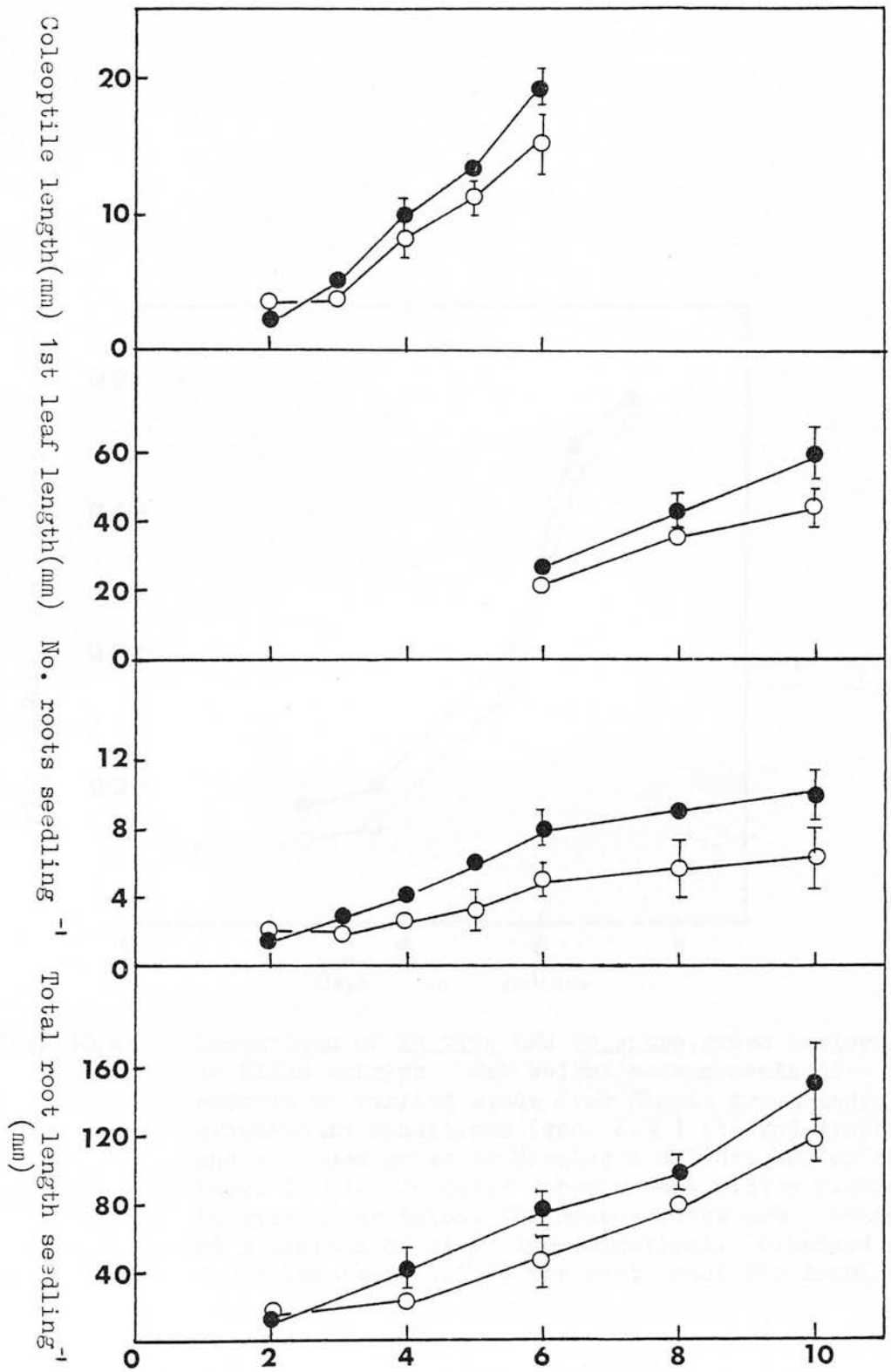


Fig. 10.3. Germination of <u>in vivo</u> and <u>in vitro</u> grown 'Midas' embryos. The rate of shoot and root development of excised mature embryos (35 days old), (closed symbols); and $< 0.5mm^2$ cultured embryos, (open symbols) germinated on Norstog's Medium II (0.1M sucrose). In the cultured embryos T_o is taken fron the onset of germination. The measurements are a mean ± Standard deviations of a minimum of ten determinations.

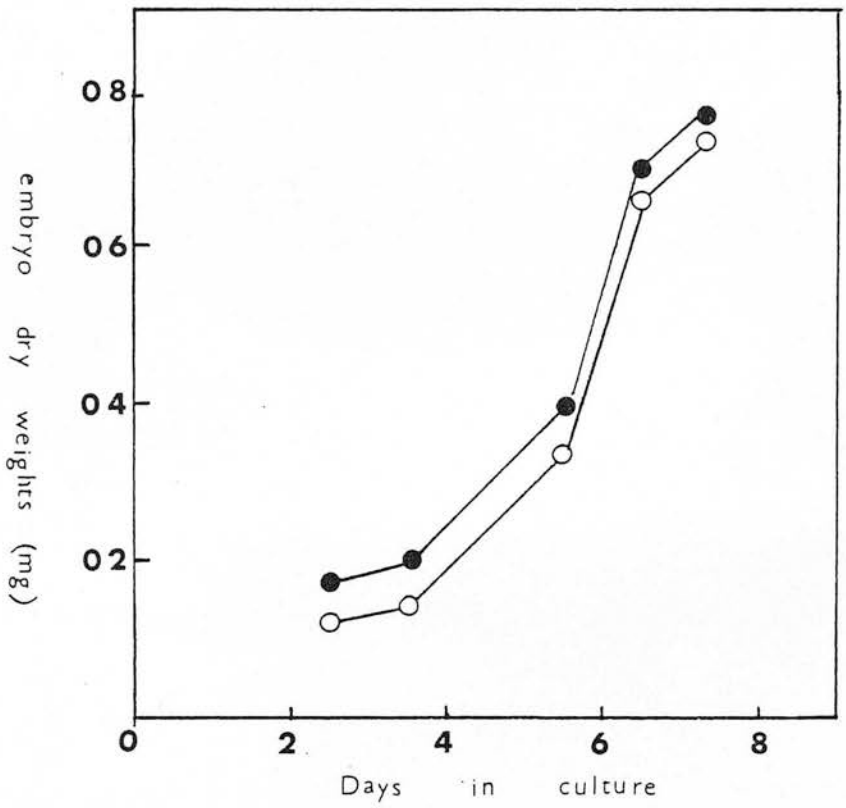


Fig. 10.4. Comparison of <u>in vivo</u> and <u>in vitro</u> grown barley cv Midas embryos. Dry weight measurements of embryos at varying sizes from plants grown under green-house conditions (sec. 2.2) (closed symbols) and of those grown in Norstog's culture Medium II (sec. 2.11). Cultured embryos were 0.55 x 0.40mm in size at excision. The measurements are means of a minimum of eight determinations. Standard deviations were all <5 per cent about the means.

Comparison of *in vivo* and *in vitro* development: Ultrastructure

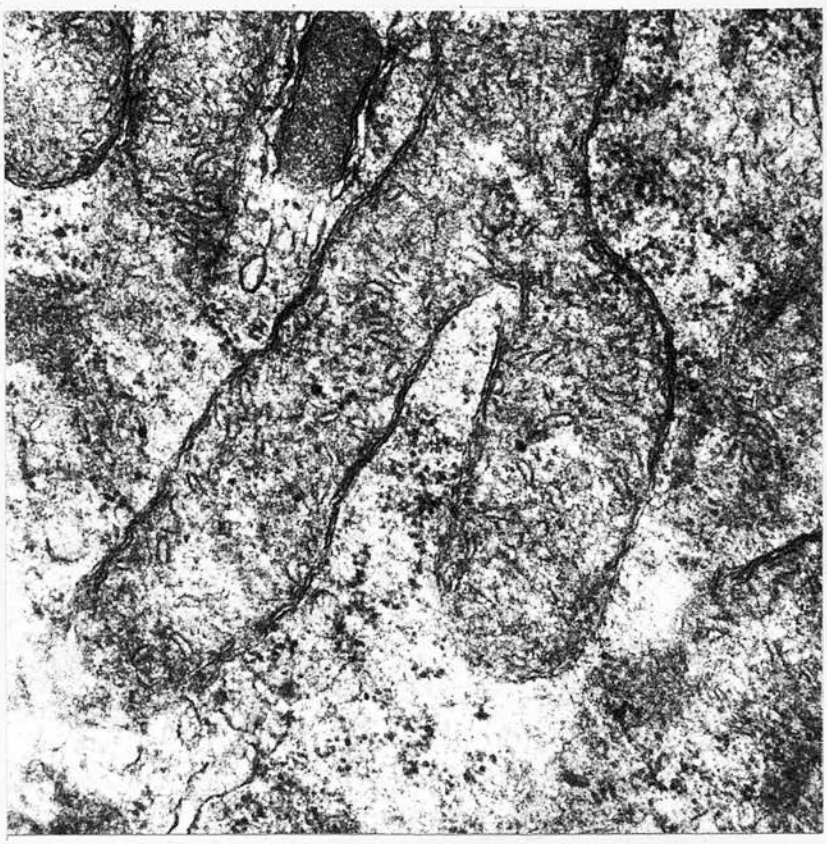
Abbreviation for Plates 10.3.1 - 10.3.6

A = Amyloplast	at = Anastomosing tubules
L = Lipid body	er = Endoplasmic reticulum
PB= Protein body	is = Intercellular space
P = Plastid	m = Mitochondrion
S = Starch	n = Nucleus
	pr = Poly-ribosomes
	r = Ribosomes
	v = Vacuole

Plate 10.3.1 Mitochondria in cells adjacent to epidermis of root region of an *in vitro* cultured embryo (size. 7.0 mm^2). X 36,000.

Plate 10.3.2 Numerous mitochondria in cells from root of an embryo (size, 7.0 mm^2) of barley cv. Midas, grown in sand culture in the greenhouse. X 24,000.

10.3.1



10.3.2

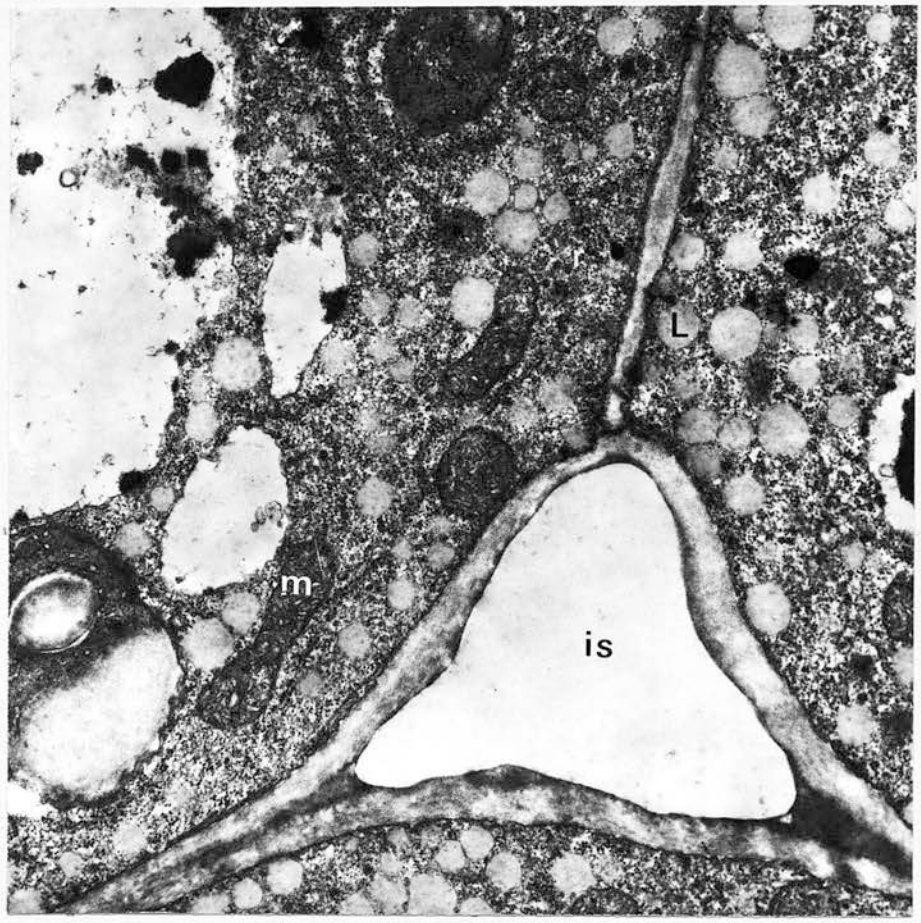
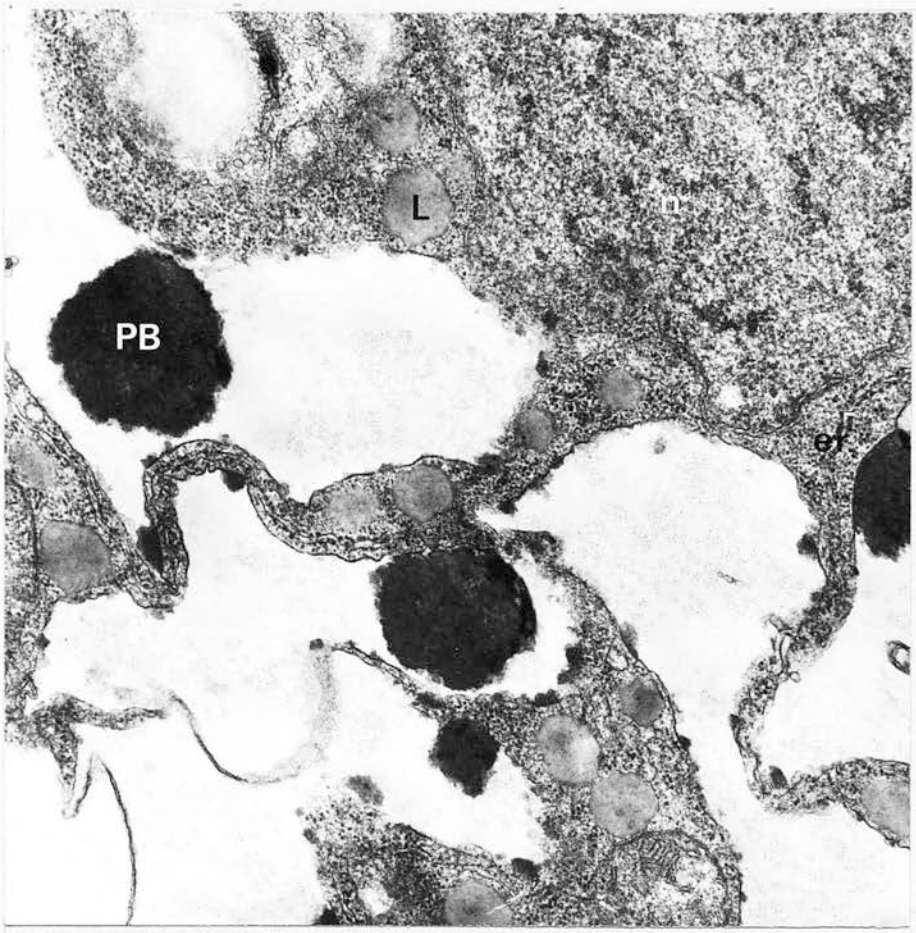


Plate 10.3.3 Lipid bodies, poly-ribosomes and endoplasmic reticulum in cells from root of an *in vitro* cultured embryo (size, 7.0 mm^2). X 24,000.

Plate 10.3.4 Lipid bodies, poly-ribosomes and endoplasmic reticulum in cells from root of embryo (size, 7.0 mm^2), of barley cv. Midas, grown in sand culture in the greenhouse. X 16,000.

10.3.3



10.3.4

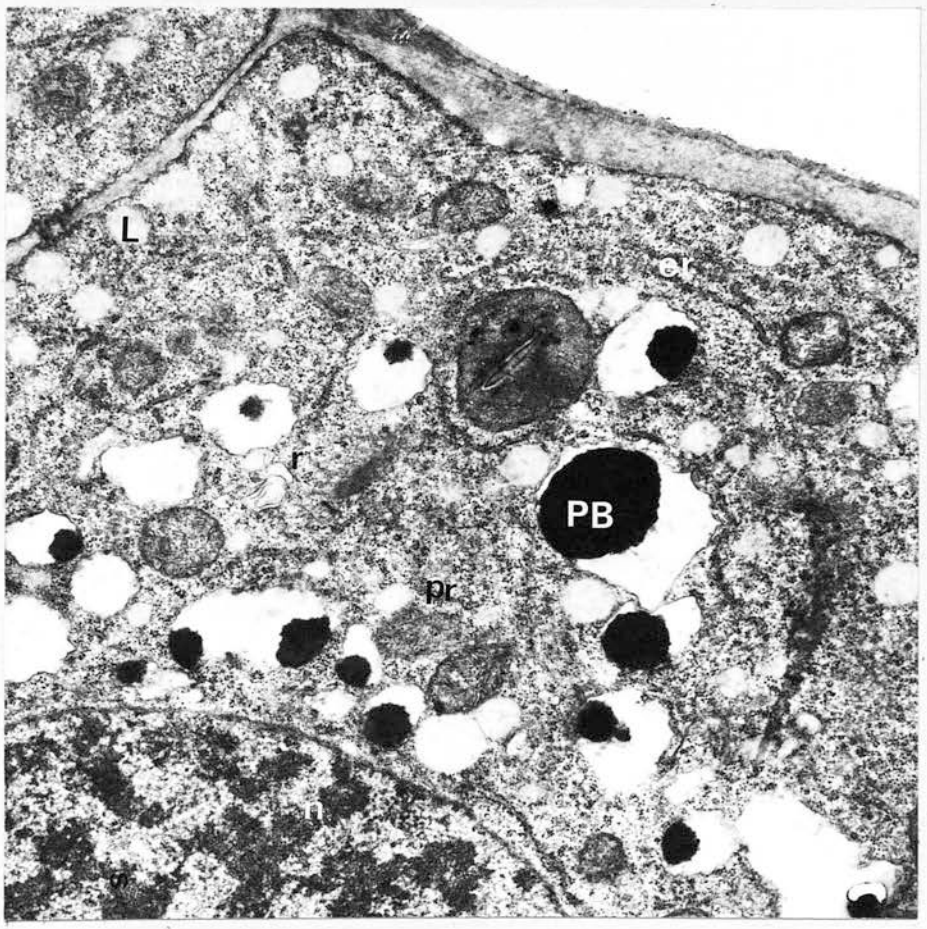
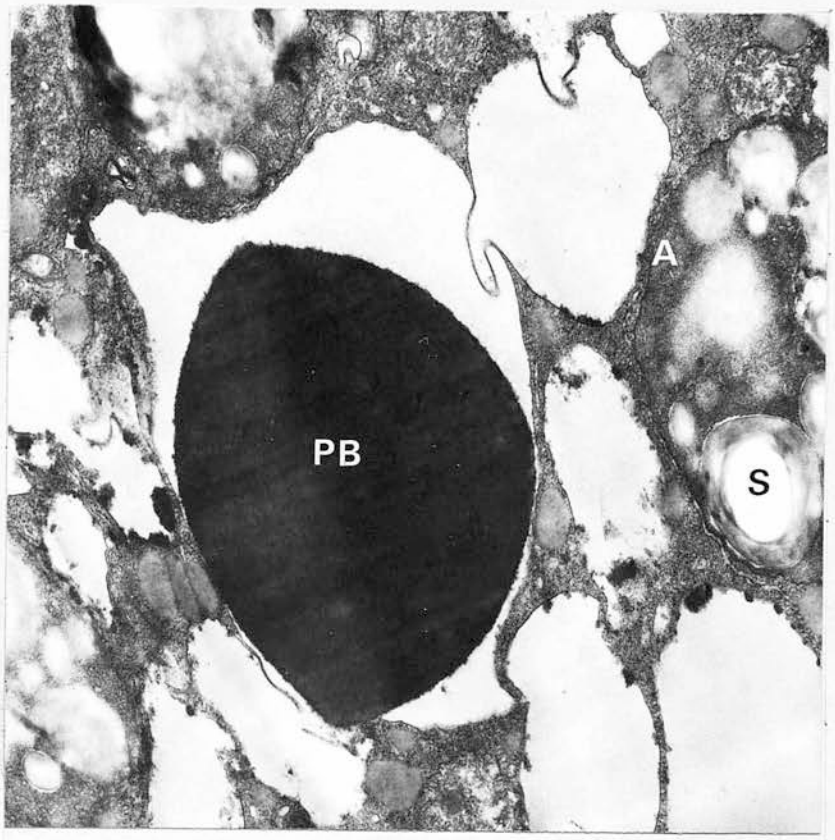


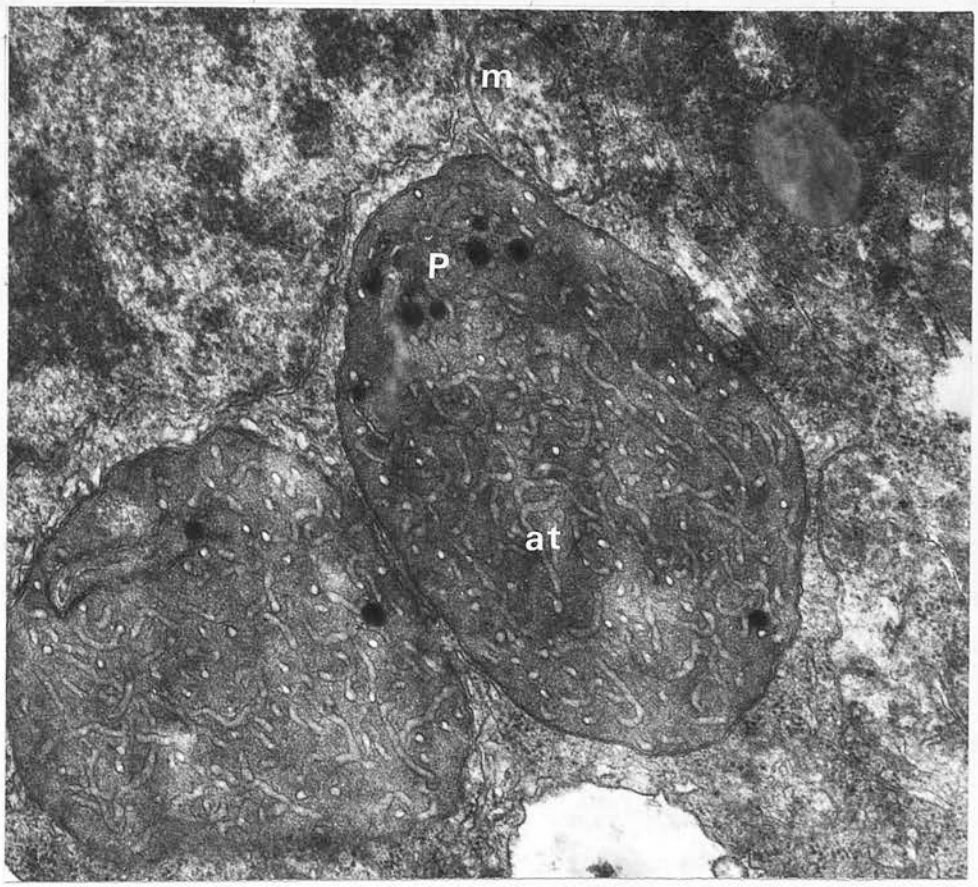
Plate 10.3.5 Protein body in vacuole and amyloplasts with starch in cells of root of an *in vitro* cultured embryo (size, 7.0 mm^2). X 15,000.

Plate 10.3.6 Plastids containing numerous anastomosing tubules in cells of root of an *in vitro* cultured embryo (size, 7.0 mm^2). X 33,000.

10.3.5



10.3.6



probably amyloplasts, which contained large numbers of anastomosing tubules in *in vitro* grown embryos. Similar tubular structures were also seen in *in vivo* grown embryos (Plate 10.3.2), but were present to a greater extent in *in vitro* embryos. A number of mitochondria were found to surround the amyloplasts (Plate 10.3.2). Protein bodies in vacuoles were present in both types of embryo cells (Plates 10.3.3, 103.5, and 10.3.4) and small grey, uniform bodies, lipid bodies were also observed (Plates 10.3.3, 10.3.4).

10.3.3 The effects on growth of sucrose concentration in the medium

The growth of cultured embryos in 0.1M and 0.2M sucrose-containing media is shown in table 10.1. When cultured on a medium containing 0.1M sucrose, embryos 0.38mm^2 and 0.23mm^2 in size at excision, germinated precociously and shoot elongation was followed by appearance of roots, at an embryo size of 4.0mm^2. In contrast, those on media containing 0.2M sucrose grew to a maximum size of 9.0mm^2 when they were transferred to test tubes for germination.

10.3.4 Growth of embryos on media containing no added Mg

The increase in growth rate of embryos cultured in media containing no added Mg(-Mg) is shown in fig. 10.5.. Embryos 0.23mm^2 in size at excision attained a size of 6.8mm^2 in 7 days on a -Mg medium, whilst those of similar average size, in a medium with added Mg (+Mg), attained a size of 3.2mm^2 in 7 days. Older embryos 0.34mm^2 in size at excision, showed a smaller difference in rates of growth (Fig. 10.5.2.) and those 0.38mm^2 in size showed no significant difference in rates of growth (Not shown).

Table 10.1: The effect of varying sucrose concentration of the medium on the growth of barley cv. Midas embryos in culture. The measurements represent the mean ± standard deviation derived from a minimum of ten determinations.

	Norstog's Medium II 0.1M sucrose		Norstog's medium II 0.2M sucrose	
Size of embryo at excision (lxw) mm^2	0.23 ± 0.01	0.38 ± 0.02	0.22 ± 0.02	0.38 ± 0.03
Size of embryo at ger mination (lxw) mm^2	3.12 ± 0.15	4.0 ± 0.50	7.8 ± 0.51	8.3 ± 0.80

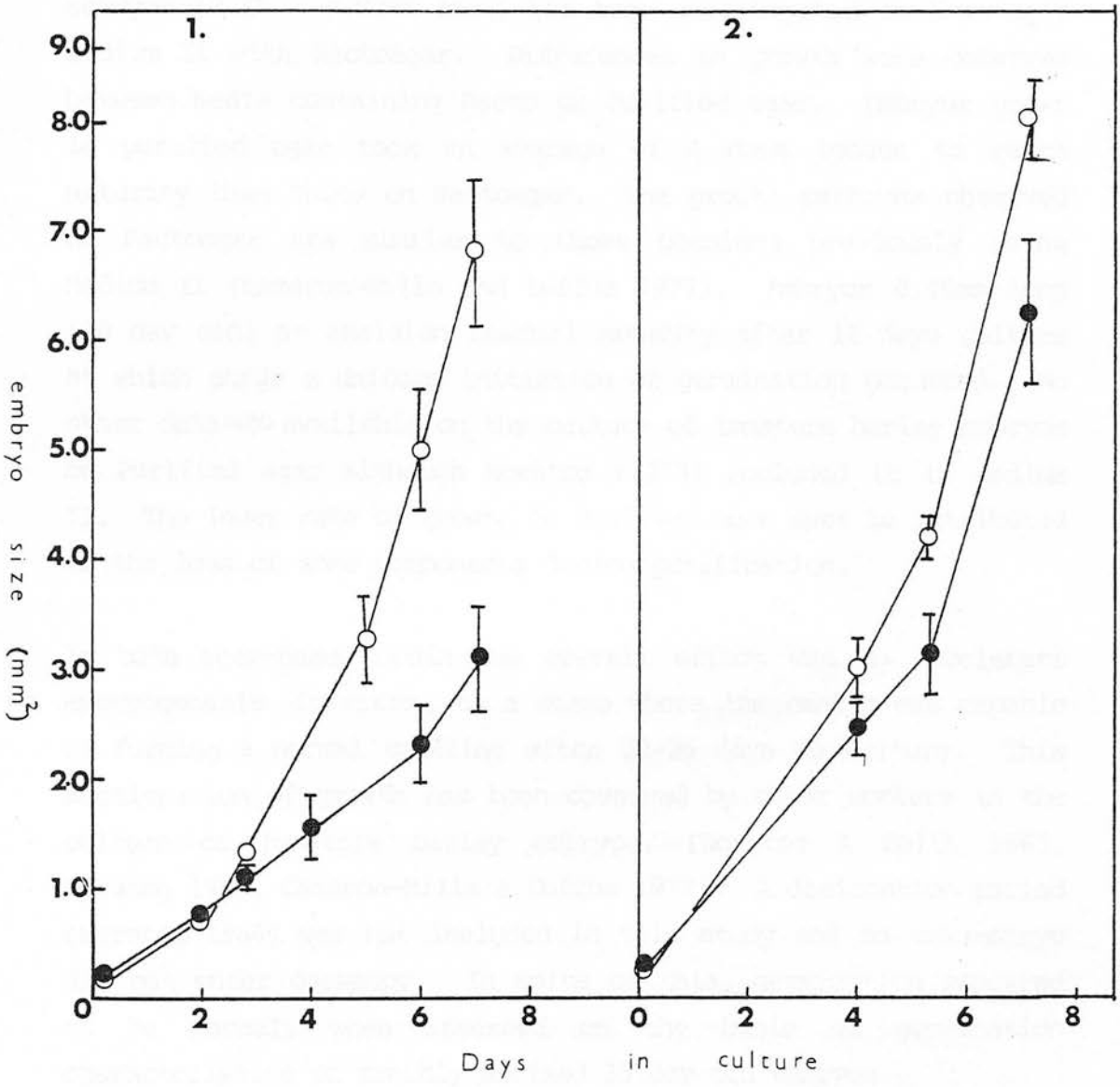


Fig. 10.5. The effect of variation in Mg supply in <u>in vitro</u> culture medium on embryo growth rates. Midas embryos of two sizes 1. 0.55 x 0.40mm and 2. 0.75 x 0.50 mm were grown on Norstog's Medium II containing the normal Mg concentration (closed symbols) and on medium where no Mg was added (open symbols). The measurements are means of a minimum of ten determinations with standard deviations about the means.

Discussion

The growth in culture of immature Hordeum distichum lam.cv.Midas embryos (0.45 - 0.83mm long) has been investigated on Norstog's medium II with Bactoagar. Differences in growth were observed between media containing Bacto or Purified agar. Embryos grown in purified agar took an average of 4 days longer to reach maturity than those on Bactoagar. The growth patterns observed on Bactoagar are similar to those obtained previously using Medium II (Cameron-Mills and Duffus 1977). Embryos 0.45mm long (10 day old) at excision reached maturity after 12 days culture at which stage a uniform initiation of germination occurred. No other data are available on the culture of immature barley embryos on Purified agar although Norstog (1973) included it in medium II. The lower rate of growth on Purified agar must be attributed to the loss of some components during purification.

In both agar-based media the overall effect was to accelerate embryogenesis *in vitro*, to a stage where the embryo was capable of forming a normal seedling after 22-26 days in culture. This acceleration of growth has been observed by other workers in the culture of immature barley embryos, (Norstog & Smith 1963, Norstog 1965, Cameron-Mills & Duffus 1977). A desiccation period (Norstog 1966) was not included in this study and so the embryo did not enter dormancy. In spite of this, germination appeared to be normal, when assessed on the basis of germination characteristics of freshly excised 35 day old embryos.

The results also suggest that embryo synthetic capacity was developed from a very early age. Additionally no growth inhibitors appear to be present in the embryos such that normal development was prevented. Excised embryos smaller than 0.2mm when cultured displayed abnormal development (Cameron-Mills & Duffus 1977). Thus, since older embryos deviate less from normal embryogenetic pathways, events occurring in the ovule up to the 0.35mm stage must be critical in determining embryonic form.

The role of the scutellum in germination and seedling nutrition is well known (Macleod & Palmer 1966). Little is known, however of the role of the scutellum during grain development. Nevertheless, it seems clear that the scutellum has a vital role to play in the uptake of nutrients, since immature embryos placed with no part of the scutellum in contact with the medium did not grow. This effect was not due to reduced oxygen supply to the differentiating shoot apex because growth of cultured embryos was not retarded in an atmosphere of N (Norstog 1965).

The developmental characteristics of embryos grown both *in vivo* and *in vitro* were remarkably similar. Occasionally however, embryos grown *in vitro* , if left in contact with culture media in the dark, may become very large. A similar effect primarily due to overgrowth of scutellum, has also been observed before (Norstog & Smith 1963, Norstog 1965).

An increase in size alone was found to be an imperfect development index, since a cultured embryo might double in length, but neither leaf nor root primordia might be initiated (Norstog 1965). In the present work therefore, a range of parameters was used in the assessment of development.

Photographic analysis representing external morphology was shown to be essential in the present work since the visual analysis of embryonic growth and development is rather subjective. In addition a photographic record provided a permanent and accurate record of the observed developmental changes. When compared with excised embryos grown *in vivo* , the *in vitro* grown embryos were morphologically very similar, suggesting normal development. The irregularities in the shape of the scutellum of embryos grown *in vitro* were expected, since the physical restrictions imposed by both the developing endosperm and the maternal tissue are absent under culture conditions.

The development of primary and seminal root primordia described by Merry (1941) in xylene cleared, camera lucida drawings, could not be distinguished clearly in these photographs.

There was little difference too, in the dry weight of embryos grown in culture compared to those harvested directly from the ear at different ages after anthesis. This further suggests that the culture medium used in the above investigations allowed 'normal' synthesis and deposition of embryonic material.

Cameron-Mills & Duffus (1977) used the morphology of germinating embryos to compare *in vitro* and *in vivo* development of embryos and came to the conclusion that normal development was maintained when embryos were grown on Norstog Medium II. The present investigation further confirms the above conclusion. However, although the patterns of growth of coleoptile, first leaf, root number and root length are similar in both investigations, germination was delayed by approximately 3 days. These were, however, two inherent differences in the media. These workers (Cameron-Mills & Duffus) used Bactoagar and 0.1M sucrose for growth and germination of the embryo, whilst in the present study Purified agar and 0.2M sucrose was used for growth and 0.1M sucrose for germination. The transfer from the higher to the lower sucrose concentration presumably required some time for the embryos to become adjusted.

Ultrastructural studies of embryos revealed that organelle differentiation of embryos grown *in vitro* compared well with that of *in vivo* grown embryos. In both, the cells appeared to be active in biosynthesis and in the accumulation of storage materials such as starch, protein and lipid.

The anastomosing tubules in the amyloplasts observed both *in vitro* and *in vivo* grown embryos confirmed previous observations, where groove reticulum (Jenkins *et al.* 1974) and thylakoid membranes (Buttrose 1960, 1963) were reported in starch-containing plastids of developing wheat and barley endosperms. This system presented a large surface area of membaranous material within an enclosed space and it was suggested by Jenkins *et al.* (1974) that this may be the site of active starch formations in the plastid. The anastomosing tubules were present to a greater extent in *in vitro* grown embryos than in embryos grown *in vivo*. Why this is so, is not clear, but the unlimited supply of sucrose to the developing embryo in culture compared to a possibly more limited supply under normal growth conditions, may lead to the formation of a more complex membraneous system to cope with the increased synthesis of starch.

Apart from this difference in amyloplast internal structure, the ultrastructure of *in vitro* embryos was similar to that of *in vivo* embryos and this has been discussed further in Sec. 11.

In the present work it was found that 0.1M sucrose (Norstog 1973, Cameron-Mills & Duffus 1977) was unsuitable for the culture of immature Midas embryos. Precocious germination was observed with all the embryo sizes cultured. Hence the sucrose concentration was doubled from 0.1M to 0.2M to overcome precocious germination. Other workers (sec 1.9.2) have previously used high sucrose concentrations e.g. 12.5 per cent (0.37M) to prevent immature embryos in culture from forming seedlings.

The pH of the endosperm bathing the embryo *in vivo* in this investigation was found to be maintained at a fairly constant level of 6.7 throughout grain development. This was in marked contrast to the much lower pH5 generally used in embryo culture (sec. 1.9.3.3). The nutritional properties of the medium or endosperm supplying the growing embryos, whether *in vivo* or *in vitro* all depend on the pH. For instance, the solubilities and availabilities of mineral ions differ e.g. Fe is soluble at

acid than at alkaline pH. Similarly the solubilities of amino acids, which depend in turn on their isoelectric points, also differ and hence their relative availabilities are altered. Furthermore, the equilibrium constants of most metal ion-organic acid complexes may be changed. It is therefore difficult to understand the use of low pH in the culture of immature embryos.

Midas embryos in comparison to the other cultivars studied previously in this laboratory (Cameron-Mills & Duffus 1977) proved even more exacting in their growth requirements. This may be because they are significantly smaller at all stages of grain development.

Since the magnesium sulphate, withdrawn from the medium in the -Mg experiments, was not replaced by another salt, it is possible that the increase in embryo size may not be a direct effect of Mg withdrawal, but rather a result of cation-anion imbalance in the Mg depleted culture medium. Nevertheless the effect correlated well with that observed in yeast (Walker & Duffus 1979) where cells grown in the absence of Mg were observed to elongate and reach twice the normal length without cell division. It is not clear, however, from the results presented here, whether or not the increased embryo size is due to an increase in cell number cell size or cell volume. In addition, the response of a multicellular organism to changes in the growth environment may differ from that of a unicellular organism.

The smaller the embryos at excision, the greater was the effect of Mg withdrawal. The effect decreased as the embryos increased in size. Hence, smaller embryos were more vulnerable and highly sensitive to their enironment. This is in agreement with other results where pro-embryos deviated frequently from normal embryogenetic pathways (Norstog 1965).

The effects described above were not always easy to reproduce, probably because of difficulty in obtaining a culture medium adequately depleted of Mg. The Mg content of even analytical grade chemicals can vary from batch to batch and ideally the mineral ion content of each complete culture medium should be analysed before use in embryo growth experiments especially where mineral ion concentrations are important.

Thus Norstog's Medium II with a sucrose concentration of 0.2M was found to support satisfactorily the growth of barley embryos in culture, as assessed by morphological criteria, germinative growth, dry weight accumulation and ultrastructural investigations. The effect of variation in Mg concentration of the medium on embryo growth showed that embryo size increased in the absence of Mg. Whether this is due to accelerated cell division or increase in cell volume is not yet clear.

11.0 Ultrastructural changes during barley embryo development.

11.1 Introduction

The changes in fine structure which occur during embryogenesis in cereals have received very little attention. Several workers (Jensen 1965, Jensen & Fisher 1967, Schulz & Jensen 1968, Bain & Mercer 1966, Marinos, 1970, Mollenhauer & Totten 1971) have reported these changes in other species, including cotton and pea. Schulz & Jensen (1968) studied the changes in ultrastructure of the Capsella embryo from the terminal cell of the 3 celled embryo through the globular stage. Cell size and number of organelles per cell decreased with repeated division through the formation of globular embryo.

The fine structure during early development of barley embryos (up to 20 celled embryo) was examined by Norstog (1972). Post-fertilisation shrinkage did not occur and plasmodesmata were not observed in cell walls of the zygote and outer cell walls of embryos. He also reported an increase in numbers of ribosomes and mitrochondria during early embryogenesis, and a reduction in mitochondrial dimensions. Similarly, Hallam (1972) examined the ultrastructural changes in development of rye embryos, but extended his study upto mature dehydrated embryos. The young embryo cells contained highly differentiated organelles, but these became less complex during maturation and dehydration.

Other workers (Setterfield *et al*.1959, Swift & O'Brien 1972, Öpik 1972) have studied the fine structure of mature pea, wheat and rice embryos, whilst others (Bain & Mercer 1966, Briarty *et al*.1970, Yoo 1970) have studied the changes during germination.

Ultrastructural studies could provide a basis for relating mineral ion accumulation patterns and other biochemical findings to cytological changes *in vivo* . There are no reports apart from Norstog's (1972) on such changes during the development of barley embryos. In this section, the ultrastructural changes occurring in barley embryos were investigated at two stages of development.

11.3 Results

Throughout this study attention was confined to the root region. At about 11 days after anthesis, the developing embryo was composed of thin walled small cells, many of which contained large vacuoles (Plate 11.1). The cytoplasm was dense, containing numerous ribosomes and mitochondria with few cristae (Plate 11.2). Lipid bodies (Plates 11.1, 11.2) and plastids, some of which contained small starch grains (Plate 11.3) were also observed. At this stage more lipid bodies were present than amyloplasts. No protein reserves were detected at this stage of development and there was little evidence of structures such as rough endoplasmic reticulum and Golgi apparatus.

At about 27 days after anthesis, the developing embryo was composed of bigger cells with thicker cell walls (Plate 11.4). The numerous mitochondria contained many cristae (Plate 11.5) and were smaller than those in the 11 day embryo. The very numerous ribosomes were present as polysomes and the rough endoplasmic reticulum was extensive (Plate 11.5 and 11.6). There was much accumulation of storage products. Lipid bodies and amyloplasts, were readily distinguishable Plates 11.4,11.5 and 11.7. The amyloplasts contained tubular structures and osmiophilic globules. Numerous electron-dense protein bodies contained within vacoules were also observed (Plate 11.6).

Ultrastructural changes during barley embryo development.

Abbreviations for Plates 11.1 - 11.7.

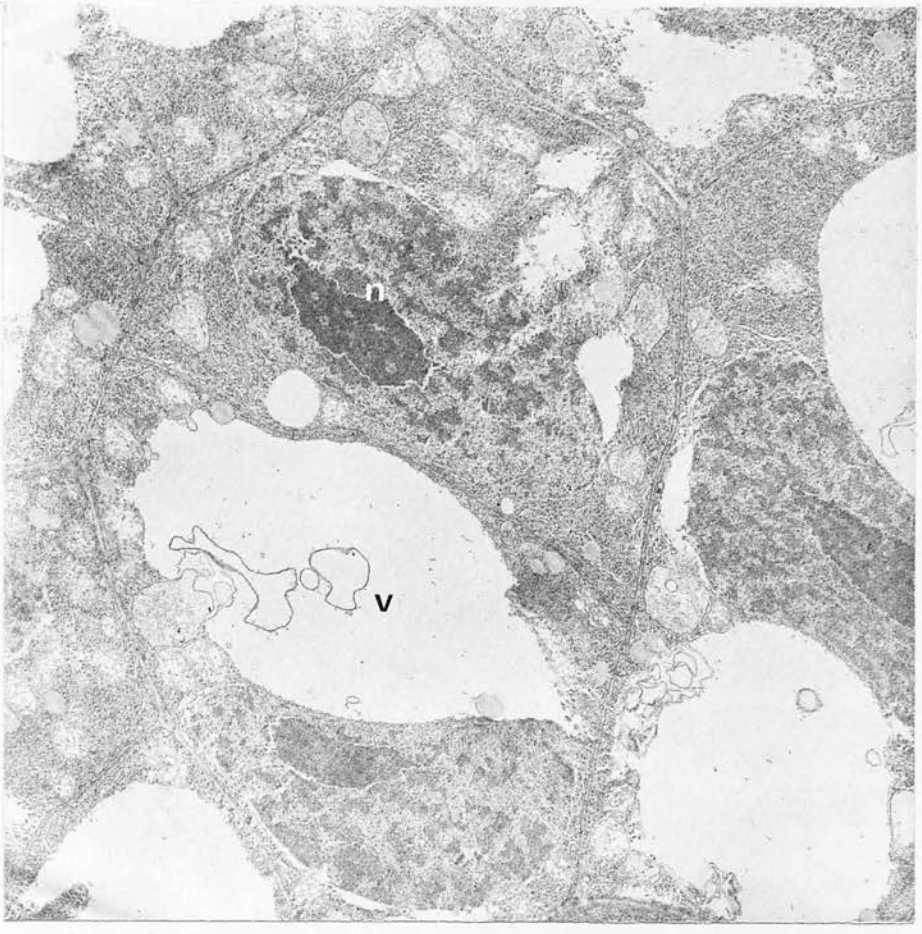
A = Amyloplast
L = Lipid body
PB= Protein body
P = Plastid
S = Starch

at = Anastomosing tubules
er = Endoplasmic reticulum
is = Intercellular space
m = Mitochondrion
n = Nucleus
pr = Poly-ribosomes
r = Ribosomes
v = Vacuole

Plate 11.1 Cells from central region of the root of 11 day old embryo from greenhouse grown barley cv. Midas. X 6,000.

Plate 11.2 Lipid bodies and mitochondria in cells of root region of 11 day old embryo from greenhouse grown barley cv. Midas. X 33,000.

11.1



11.2

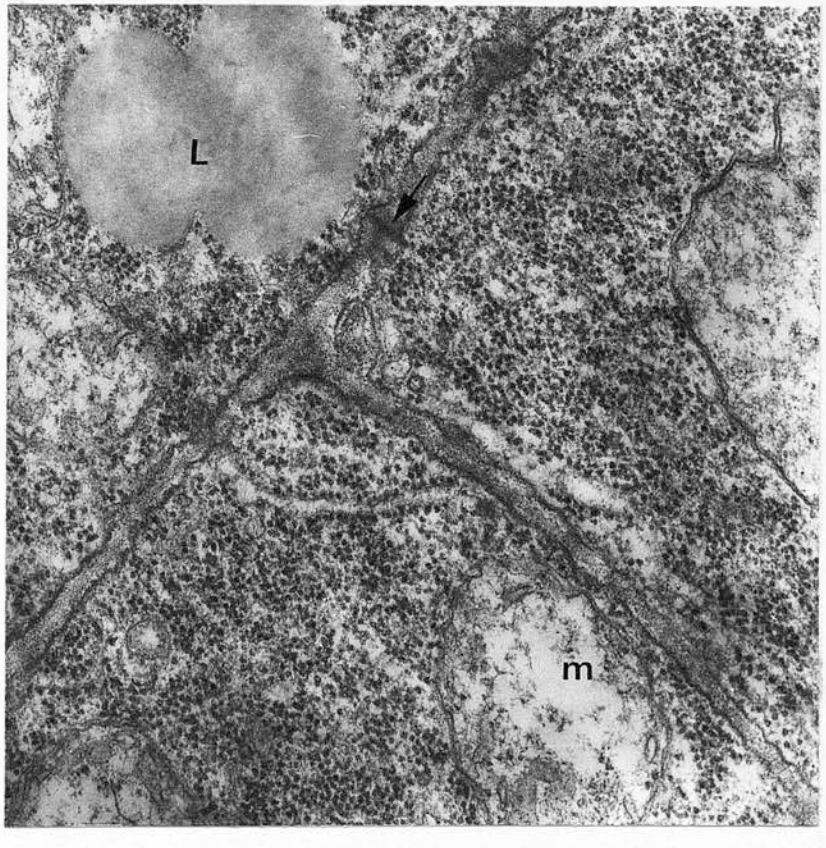
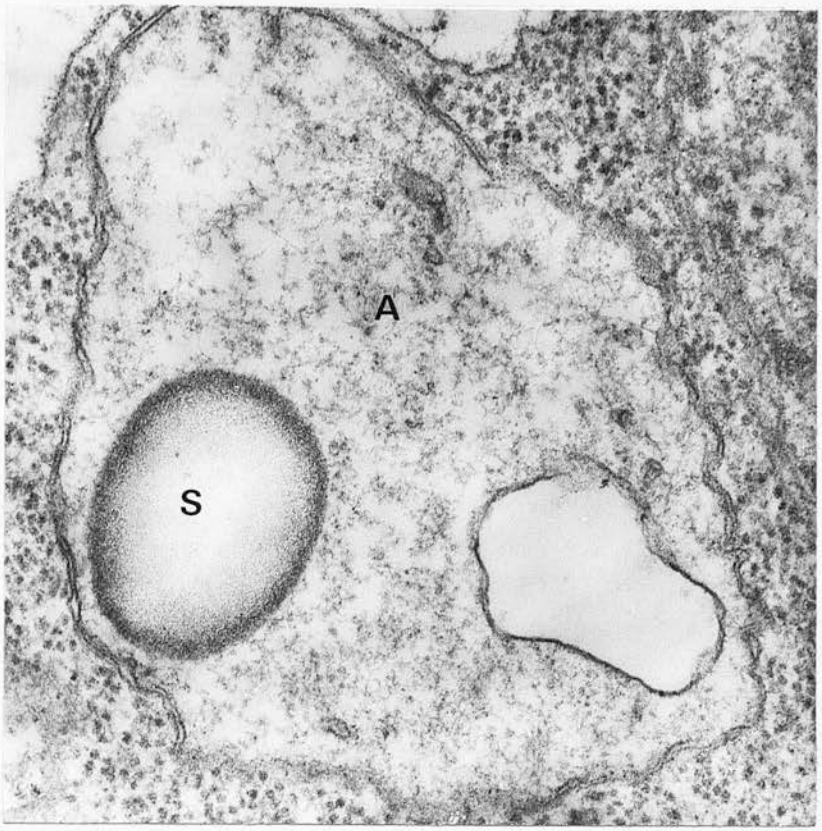


Plate. 11.3 Amyloplast containing starch in cells of root region of 11 day old embryo from greenhouse grown barley cv. Midas. X 60,000.

Plate 11.4 Transverse section of root of 27 day old embryo from barley cv. Midas, grown in sand culture in the greenhouse. X 3,000.

11.3



11.4

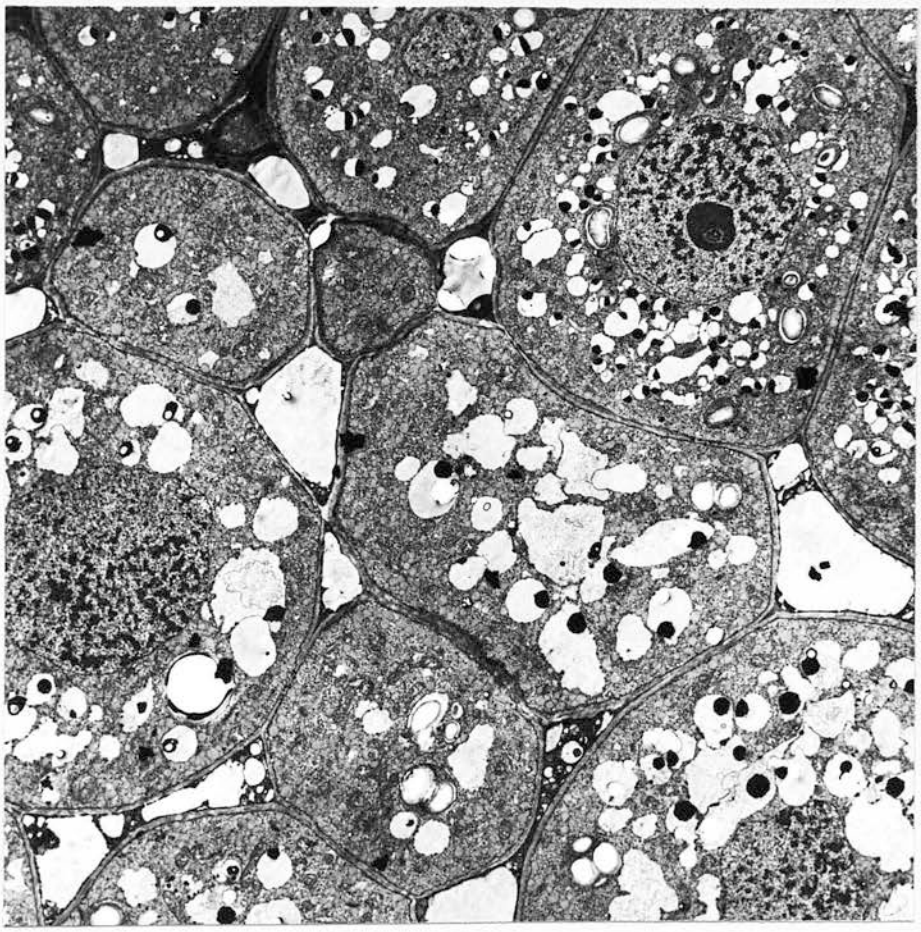
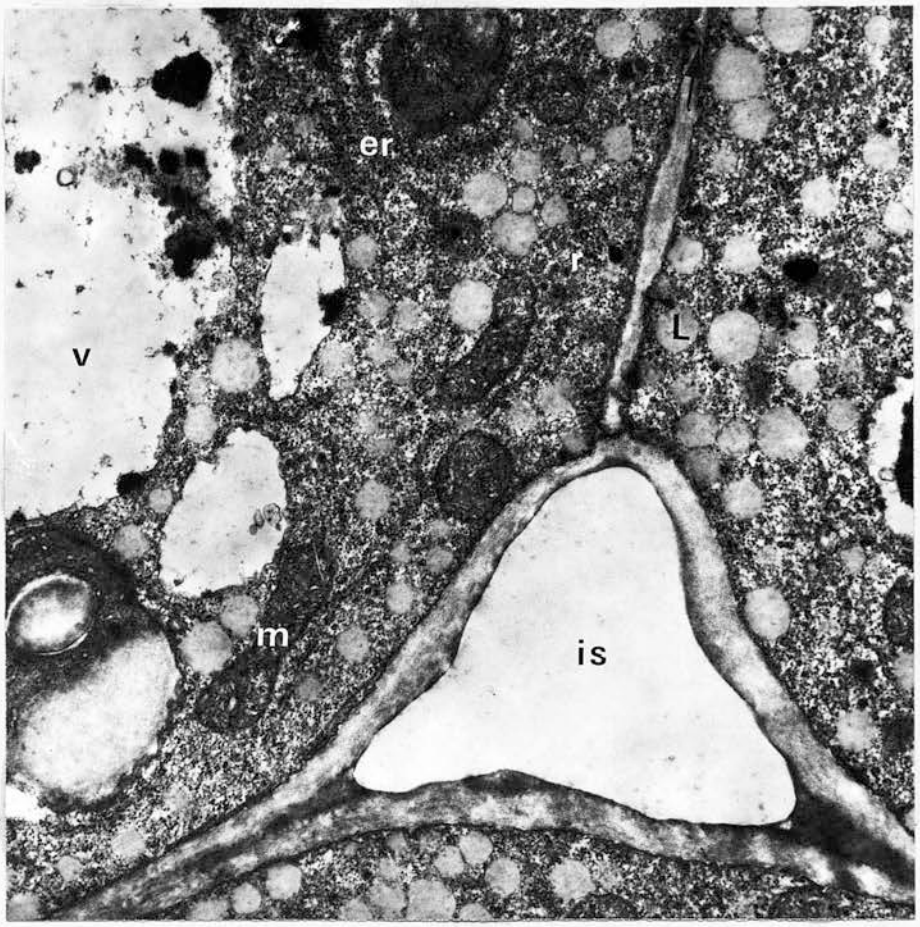


Plate 11.5 Cells from the central region of the root of 27 day old embryo from barley cv. Midas, grown in sand culture in the greenhouse. X 16,000.

Plate 11.6 Outer wall with cuticular layer (➹) of epidermal cell from the root of 27 day old embryo of barley cv. Midas, grown in sand culture in the greenhouse. X 16,000.

Plasmodesmata (→)

11.5



11.6

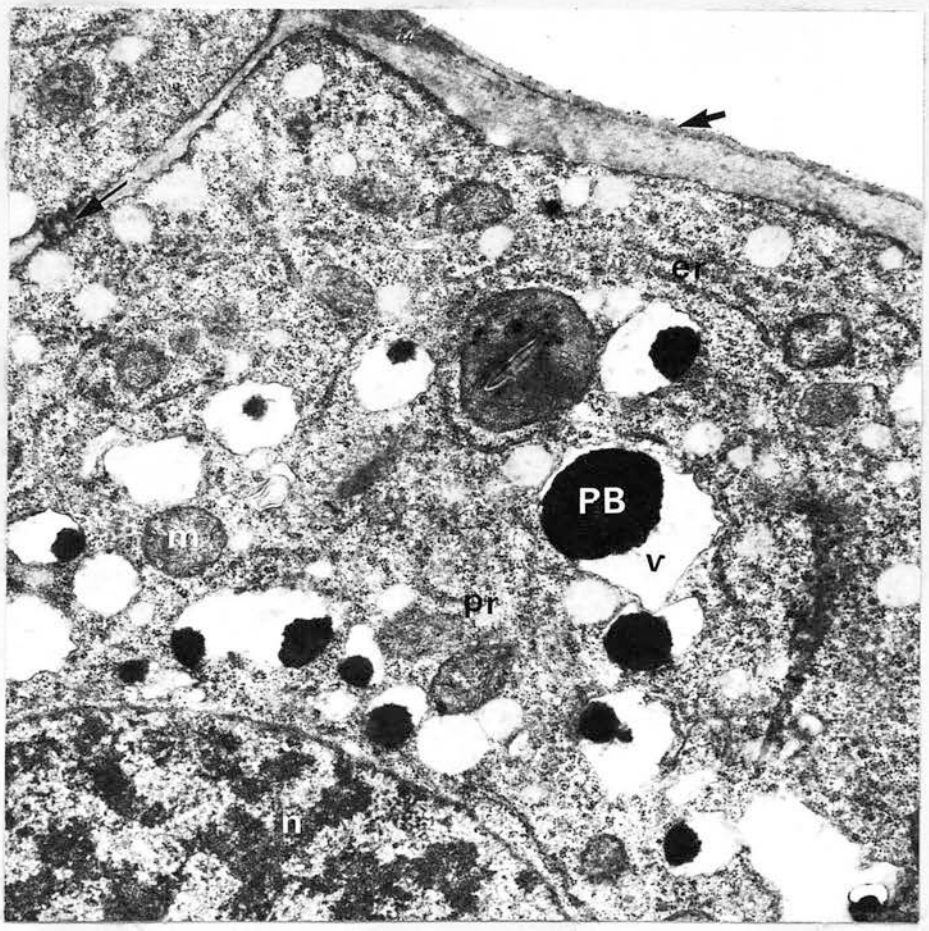
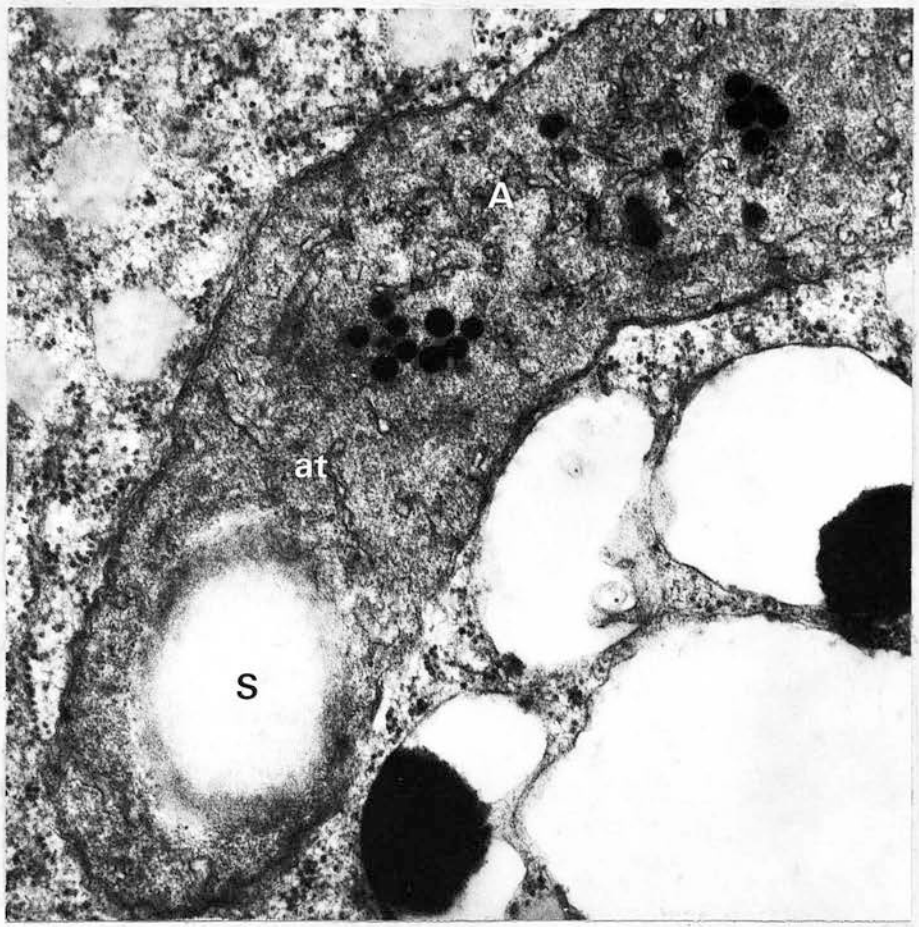


Plate 11.7 Amyloplast and protein bodies in the epidermal cell of 27 day old embryo from barley cv.Midas, grown in sand culture in the greenhouse. X44,000.


A
at
S

Plasmodesmata were present at both stages of development (Plates 11.1,11.2, 11.4, 11.5, 11.6). but were not observed in the outer wall of the epidermis. This outer wall was surrounded by thin cuticular layer (Plate 11.6).

Discussion

Even at the early stages of embryogenesis many organelles could be seen. The presence of numerous ribosomes indicated that the cells were very active. However, a large portion of each cell was occupied by large vacuoles. Similar, large vacuoles have been described in cells of young wheat scutellum and in 20 celled barley embryos (Hršel *et al.*1961, Norstog 1972).

The lipid bodies were found to be distributed throughout the cytoplasm in the younger embryos, but in the older embryos these tended to accumulate close to the plastids and the plasma membrane. These bodies have been observed both in young and mature plant embryos, (Setterfield *et al.*1959, Nieuwdorp 1963, Rost 1970, Mollenhauer & Totten 1971 and Hallam 1972). The reason why the lipid accumulates so close to the plastids and plasma membranes is not clear. During the last stages of dehydration of rye embryos, lipid bodies were observed to be packed against the plasma membrane (Hallam 1972). This situation has been associated with metabolically inactive tissue (Yoo 1970, Abdul-Baki & Baker 1970). These bodies may not be organelles in the structural sense, since although they appear to be bounded by a single membrane, Frey-Wyssling *et al.*(1963) have pointed out that an oil and protein surface can appear as an electron dense membrane in the electron microscope.

The amyloplasts were bounded by a double membrane, and resembled those observed in developing cereal endosperm (Duffus 1979). The tubular structures previously described (sec 10.3.2.4) in the amyloplasts of 27 day old embryo, were not as evident in the 11 day old embryo.

Biochemical investigations on the developing barley embryo revealed that the younger embryos contained a higher carbohydrate than protein content (Duffus & Rosie 1975). No protein bodies were observed in the 11 day old embryos, although, in contrast, both amyloplasts and lipid bodies were prevalent. Protein reserves were present in the older embryos. Similarly protein reserves were only detected in 22 day old pea embryos whilst starch grains were visible much earlier (Bain & Mercer 1966).

The protein bodies in the 27 day barley embryos were more electron dense than those observed in the starchy endosperm (Rost 1972), but similar to the developing aleurone grains of the aleurone layer (Personal communication M.P. Cochrane). Cereal embryos have been shown to contain various types of protein bodies, some with inclusions and some without (Rost 1972). While much of the volume of the protein bodies is occupied by proteinaceous reserves, the protein bodies are also responsible for storage of most of the seed's supply of minerals. Mineral reserves have been reported to occur mainly within the electron-dense globoid crystals observed in some protein bodies (Lott 1975, Ogawa *et al.* 1975,) . . . . . and are mainly present as a cationic salt of phytic acid (sec.1.6.6). Very few of the mineral reserves of the endosperm are found in the starchy endosperm, almost all being present in the aleurone layer.

The apparent differences in the organisation of ribosomes at the two stages of development indicated that two populations of ribosomes may occur in the cytoplasm. One group was concerned with the synthesis of the enzymic equipment of the meristematic and enlarging cells, and the other with the synthesis of storage protein and of the enzymes involved in protein and starch synthesis. If plastids are self-duplicating organelles, however, the enzymes of starch synthesis may be independent of the ribosomes of the cytoplasm (Bain & Mercer 1966).

The mitochondria were usually relatively small both in the young and older embryo although they were widespread within the cell. Small mitochondria (0.25 - 0.5 μ diameter) were also observed in cells of both mature wheat and pea embryos (Setterfield *et al.* 1959). The presence of mitochondria with many cristae may indicate that the developing embryos respire aerobically. However, in experiments on anaerobically grown rice coleoptiles it was shown that mitochondria were not only abundant but that the crista density was only slightly lower than in aerobically grown cells. Further, this high crista density was associated with very much depressed cytochrome oxidase activity. It was pointed out that no obligate correlation between crista density and intensity of oxidative metabolism existed in this tissue (Öpik 1973).

Nevertheless, if the developing embryo is an aerobe, the oxygen supply of the embryo may be limited due to the presence of a cuticular layer on the outside of the embryo epidermal cells. Cochrane & Duffus (1979) have pointed out that the cuticles observed in the testa-pericarp region probably impede the movement of oxygen and hence the availability of oxygen within the developing grain may be restricted.

The requirement for mineral ions throughout embryo development can be explained by the very high intracellular complexity, indicating much synthetic activity even at the very early developmental stages. Protein bodies probably store the minerals accumulated at later stages of development when metabolic activity is low.

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